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Significance of hepatitis B virus core-related antigen and covalently closed circular DNA levels as markers of hepatitis B virus re-infection after liver transplantation

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Abstract

Background and Aim: Currently, hepatitis B virus (HBV) re-infection after liver transplantation (LT) can be almost completely suppressed by the administration of HBV reverse transcriptase inhibitors and hepatitis B immunoglobulins. However, after transplantation, there is no indicator of HBV replication because tests for the serum hepatitis B surface antigen and HBV-DNA are both negative. Therefore, the criteria for reducing and discontinuing these precautions are unclear. In this study, we examined the serum HBV core-related antigen (HBcrAg) and intrahepatic covalently closed circular DNA (cccDNA) in order to determine if these could be useful markers for HBV re-infection.

Methods: Thirty-one patients underwent LT for HBV-related liver disease at Nagasaki University Hospital from 2001 to 2010. Of these, 20 cases were followed up for more than 1 year (median follow-up period, 903 days). We measured serum HBcrAg and intrahepatic cccDNA levels in liver tissue. In addition, in nine cases, we assessed the serial changes of HBcrAg and intrahepatic cccDNA levels from preoperative periods to stable periods.

Results: We examined serum HBcrAg and intrahepatic cccDNA levels in 20 patients (35 samples). HBcrAg and cccDNA levels were significantly correlated with each other ($r = 0.616$, $P < 0.001$). From a clinical aspect, the fibrosis stage was significantly lower in both HBcrAg- and cccDNA-negative patients than in HBcrAg- or cccDNA-positive patients.

Conclusions: HBcrAg and cccDNA were useful as HBV re-infection markers after LT. Keeping patients' HBcrAg and cccDNA negative after LT might contribute to long-term graft survival.

Authors' Contributions:
Toshihisa Matsuzaki: acquisition of data, study concept and design, statistical analysis, writing of manuscript.
Tatsuki Ichikawa: study concept and design, acquisition of data, critical revision of the manuscript for important intellectual content.
Masashi Otani: critical revision of the manuscript for important intellectual content.
Motohisa Akiyama: critical revision of the manuscript for important intellectual content.
Eisuke Ozawa: critical revision of the manuscript for important intellectual content.
Satoshi Miuma: critical revision of the manuscript for important intellectual content.
Sadayuki Okudaira: acquisition of data, critical revision of the manuscript for important intellectual content.
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Hisamitsu Miyaaki: critical revision of the manuscript for important intellectual content.
Susumu Eguchi: critical revision of the manuscript for important intellectual content.
Takashi Kanematsu: critical revision of the manuscript for important intellectual content.
Hajime Isomoto: critical revision of the manuscript for important intellectual content.
Fuminao Takeshima: critical revision of the manuscript for important intellectual content.
Kazuhiko Nakao: study supervision, critical revision of the manuscript for important intellectual content.

Introduction

Liver transplantation (LT) is an established procedure for the treatment of end-stage liver disease. However, the recurrence of hepatitis B virus (HBV) is implicated in life-threatening graft failure.[1] Therefore, the prevention of HBV recurrence following LT is a serious concern. The advent of hepatitis B immunoglobulins (HBIg) and the HBV reverse transcriptase inhibitor (RTI) was a major breakthrough in the management of HBV recurrence. Currently, an ideal recurrence rate for HBV has been observed in patients who received HBIg and RTI combination therapy.[2] However, several studies have reported that HBV can be detected in the transplanted liver and peripheral blood mononuclear cells of recipients even when they have a hepatitis B surface antigen (HBsAg)-negative status.[3] Therefore, prophylaxis currently must be continued for the patient's lifetime. However, there are concerns with the long-term administration of HBIg and RTI with respect to safety, medical costs, and resistant mutations of HBV.[4] In order to discontinue the prophylaxis, several groups have attempted to vaccinate LT recipients against HBV, but most of these studies involve relatively low seroconversion rates because of the immunosuppressive environment.[5]

Recently, new agents against HBV, such as adefovir and entecavir, which hardly develop resistant mutations, have become available. Some have reported that HBIg can be discontinued after LT by using the new anti-HBV agents even if the vaccination does not succeed.[6] Angus *et al.* reported that when adefovir dipivoxil was substituted for low-dose HBIg, all patients were alive at the study completion without recurrence.[7] In addition, low-risk cases, such as those with fulminant hepatitis, and hepatitis B core antibody (HBcAb)-positive donors are not necessary for the administration of high-dose HBIg.[8] However, after transplantation, RTI and HBIg may mask the appearance of HBV-DNA, regardless of the presence of intrahepatic HBV covalently closed circular DNA (cccDNA). These factors make it difficult to detect HBV dynamics following LT, and we are therefore unable to determine the feasibility of the discontinuation of prophylaxis.

Recently, a new enzyme immunoassay that detects hepatitis B core-related antigen (HBcrAg) has been reported.[9] HBcrAg changes in parallel with HBV-DNA in the serum and has a wide detection range.[10] Moreover, its levels are correlated with the intrahepatic cccDNA levels of patients with chronic hepatitis B.[11] In addition, we previously reported on the usefulness of HBcrAg in patients receiving anti-HBV prophylaxis following LT.[12]

Therefore, in this study, we simultaneously measured serum HBcrAg and intrahepatic cccDNA levels in liver tissue and studied the HBV dynamics in patients following HBV-related LTs.

Methods

Patients and samples. From 2001 to 2010, a total of 31 patients with HBV-related end-stage liver disease underwent LTs at Nagasaki University Hospital, Nagasaki, Japan. Of these, we enrolled 20 patients who could be followed up for more than approximately 1 year (median 902 days; range 323–2456 days). There were 17 men and 3 women, with a median age of 56.5 years (range 33–68 years). All 20 patients were diagnosed with liver cirrhosis, and 12 were diagnosed with hepatocellular carcinoma. In addition, two patients were coinfected with the hepatitis C virus (Table 1).

Table 1 Baseline clinical features of the enrolled patients

Case	Age	Gender	Indication disease	HBV-DNA	HBsAg	HBsAb	HBeAg	HBeAb	HBcAb	Donor HBcAb	HBcrAg
1	55	F	LC-B	< 2.6	> 2000	0.2	36.0	0.0	> 100.0	5.0	6.0
2	56	M	LC-B	< 2.6	> 2000	2.3	0.6	82.4	99.9	5.0	4.2
3	48	M	LC-B, HCC	< 2.6	562.5	0.1	1.1	57.7	> 100.0	31.3	5.0
4	60	M	LC-B	< 2.6	1789	0.1	0.2	97.6	> 100.0	70.1	5.8
5	59	M	LC-B, HCC	< 2.6	> 2000	0.1	0.1	> 100.0	> 100.0	5.0	3.2
6	57	M	LC-B, HCC	3.9	188.5	0.5	0.8	54.0	> 100.0	10.3	5.1
7	56	M	LC-B, HCC	< 2.6	> 2000	0.1	1.4	75.4	> 100.0	91.9	5.6
8	68	M	LC-B, HCC	< 2.6	> 2000	0.2	0.1	> 100.0	> 100.0	5.0	3.0
9	33	F	LC-B	3.0	> 2000	0.2	0.2	81.5	99.9	99.6	5.5
10	58	M	LC-B, HCC	3.0	> 2000	0.1	0.1	93.6	> 100.0	93.4	5.1
11	59	M	LC-B	< 2.6	378.3	0.3	0.1	61.6	> 100.0	93.0	3.8
12	57	M	LC-B + C, HCC	< 2.6	519.9	0.1	0.1	> 100.0	99.9	5.0	2.0
13	49	M	LC-B	< 2.6	> 2000	0.1	0.9	52.9	> 100.0	34.1	5.2
14	65	F	LC-B	6.9	> 2000	0.2	0.1	> 100.0	> 100.0	5.0	6.8
15	55	M	LC-B, HCC	< 2.1	> 2000	0.2	0.1	99.3	> 100.0	31.6	4.5
16	46	M	LC-B + C	4.3	1100.4	0.2	0.1	> 100.0	> 100.0	81.9	3.7
17	59	M	LC-B, HCC	< 2.1	> 2000	0.1	0.1	99.2	> 100.0	38.6	3.7
18	51	M	LC-B, HCC	2.1	> 2000	0.2	0.4	62.8	99.4	50.0	4.7
19	67	M	LC-B, HCC	3.9	> 2000	0.1	34.3	60.2	> 100.0	91.1	6.3
20	54	M	LC-B, HCC	2.1	> 2000	0.1	104.8	37.4	> 100.0	9.7	4.3

HBV, hepatitis B virus; HBcAb, hepatitis B core antibody; HBcrAg, hepatitis B core-related antigen; HBeAb, hepatitis B envelope antibody; HBeAg, hepatitis B envelope antigen; HBsAb, antibody against hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LC, liver cirrhosis; LC-B, LC due to HBV; LC-B + C, LC due to HBV-HCV coinfection.

All patients had been receiving RTI since preoperative periods. The HBsAg was negative in all donors, but eight donors were HBcAb-positive (cut-off, 50%), which was suggested to be due to prior exposures to HBV.

The prophylactic infusion of HBIg was administered to all patients according to a fixed-dose schedule; 10 000 units were given intravenously at the anhepatic period during the operation and the next day after the living donor LT (LDLT). Afterwards, 2000 units of HBIg were given routinely in order to keep the serum hepatitis B surface antibody (HBsAb) titers above 100 units/L. After the LDLT, serum HBsAg, hepatitis B envelope antigen (HBeAg), and HBV-DNA were not detected in any of the patients in this study.

Serum samples and biopsy specimens were obtained from 20 patients who received protocol biopsies 1 year after the LDLT at Nagasaki University Hospital after providing informed consent. Nine patients were followed up from the preoperative period to the stable period. Serum samples were obtained at the following three specified intervals: (i) in the preoperative period, samples were obtained just before the operation; (ii) in the postoperative period, samples were obtained during the operation of LT; and (iii) in the stable period, samples were obtained during admission for protocol biopsy. Liver tissue samples were obtained during the following three specified procedures: (i) biopsy from explanted liver during the operation; (ii) time-zero biopsy from the implanted liver during the operation; and (iii) protocol biopsy 1 year after the LDLT.

Serological markers for HBV. HBsAg, HBsAb, HBeAg, hepatitis B envelope antibodies (HBeAb), and HBcAb levels were assessed by the chemiluminescence enzyme immunoassay (CLEIA) method using a commercially available enzyme immunoassay kit (Lumipulse, Fuji Rebio, Inc., Tokyo, Japan). Serum concentrations of HBV-DNA were determined using a polymerase chain reaction (PCR) HBV monitoring kit (Roche Diagnostics K.K., Tokyo, Japan), which had a quantitative range from 2.6 to 7.6 log copies/mL.

HBcrAg test. Serum HBcrAg levels were measured by a CLEIA HBcrAg assay kit (Fujirebio, Inc.) with a fully automated analyzer system (Lumipulse System, FujiRebio, Inc.). HBcrAg concentrations were expressed as units/mL (U/mL). In this study, HBcrAg values were expressed as log U/mL, and the cut-off value was set at 3.0 log U/mL.[9,13]

Measurement of cccDNA. Liver tissues were stored at –80°C before DNA extraction. HBV-DNA was extracted using a high pure PCR template preparation kit (Roche Diagnostics K.K.). The concentration of purified DNA was measured at an absorbance of 260 nm.

cccDNA levels were measured with the real-time PCR method. With reference to a previous study,[11] we designed two oligonucleotide primers, cccF2 (5′-CGTCTGTGCCTTCTCATCTGA-3′, nucleotides: 1424-1444) and cccR4 (5′-GCACAGCTTGGAGGC TTGAA-3′, nucleotides: 1755-1737), and a cccP2 probe (5′-FAM-ACCAATTTATGCCTACAG-MGB-3′, nucleotides: 1672-1655). Reaction volume (20.0 μL) containing 500 ng of extracted DNA, 0.5 μmol/L of each primer, 0.2 μmol/L of the probes, and LightCycler TaqMan Master (Roche Diagnostics K.K.) was administered. The initial activation step was heated at 95°C for 10 min. The subsequent PCR conditions consisted of 60 cycles of denaturation at 95°C for 10 s, and annealing and extension at 60°C for 30 s per cycle. Real-time PCR was performed in a LightCycler (Roche Diagnostics K.K.). Serial dilutions of a plasmid containing an HBV monomer were used as quantitation standards.

Liver histology. Liver histology was evaluated by the same two pathologists. The degrees of necroinflammation and fibrosis were assessed based on the New Inuyama classification.[14] The degrees of rejection were assessed with the Rejection Activity Index according to the Banff working classification of hepatic allograft pathology.[15]

Liver function test. Blood biochemical tests were performed in all patients, and liver function was evaluated. Liver function was assessed using Pugh's modification of Child's scoring system.[16]

Statistical analyses. Student's *t*-tests and Fisher's exact tests were used for comparisons between groups of parametric quantitative data, and Mann–Whitney *U*-tests were used for comparisons between independent groups of non-parametric data. Categorical variables were compared with chi-square tests. The correlations between continuous variables were analyzed by the Pearson's correlation test. Two-tailed *P* values less than 0.05 were considered statistically significant.

Results

Correlation between HBcrAg and cccDNA. The correlation between HBcrAg and cccDNA levels in all 35 samples is summarized in Figure 1. A statistically significant positive

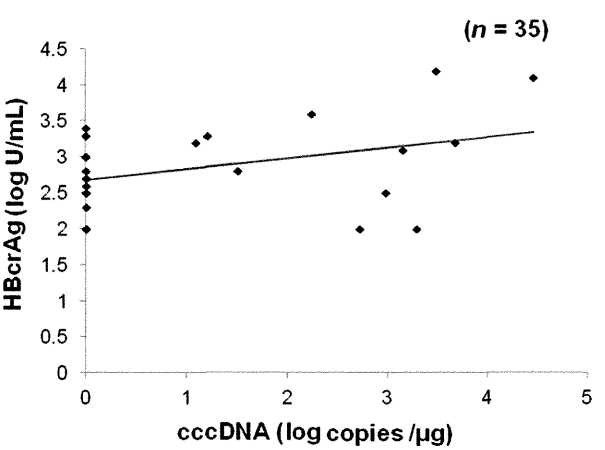

Figure 1 Correlation between serum hepatitis B core-related antigen (HBcrAg) and intrahepatic hepatitis B virus covalently closed circular DNA (cccDNA). $r = 0.616$, $P < 0.001$ ($y = 0.40x + 2.62$). Straight lines indicate the correlation between HBcrAg and cccDNA levels.

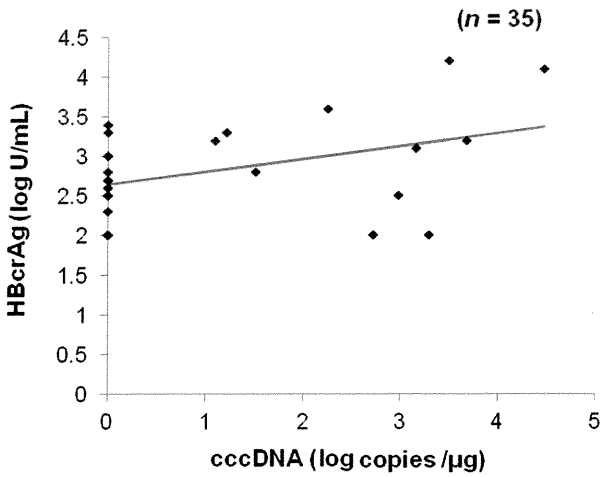

Figure 2 Correlation between hepatitis B core-related antigen (HBcrAg) and covalently closed circular DNA (cccDNA) levels after transplantation. $r = 0.402$, $P = 0.046$ ($y = 0.16 \times + 2.64$). Straight lines indicate the correlation between HBcrAg and cccDNA levels.

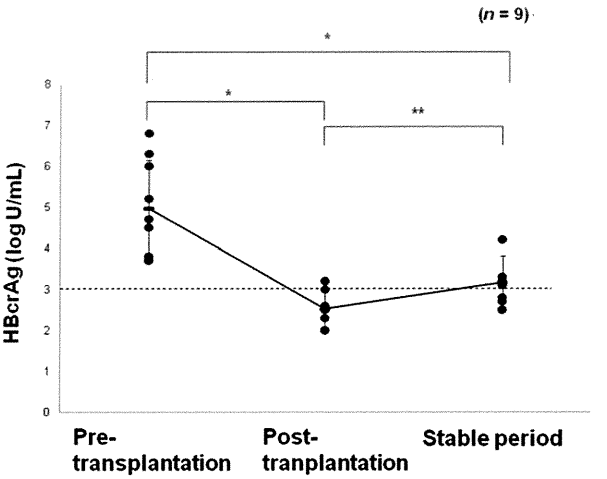

Figure 3 Serial changes of the hepatitis B core-related antigen (HBcrAg) levels. HBcrAg levels are represented as mean values; the closed circles show the values of the HBcrAg levels in all phases. The error bars indicate standard deviations. The detection range is above 3.0 log U/mL. In order to obtain the mean value, the values of 3.0 log U/mL or less, and 2.0 log U/mL or more were added to the calculation. The mean values of HBcrAg levels dropped during the postoperative period but then gradually increased again during the stable period (*$P < 0.001$ and **$P = 0.035$ indicate the significant differences between each period).

correlation was observed ($r = 0.616$, $P < 0.001$). Similarly, in the 23 samples that were obtained after LT only (that is, preoperative state samples were excluded), HBcrAg levels were significantly correlated with cccDNA levels (Fig. 2, $r = 0.402$, $P = 0.046$). These results supported the hypothesis that HBcrAg can be useful as an HBV marker instead of cccDNA after LT.

Serial changes in HBcrAg and cccDNA levels. HBcrAg and cccDNA levels showed similar dynamics during each period (Figs 3,4). All nine cases had positive levels of HBcrAg. However, seven of them were negative for HBV-DNA. During the post-transplantation period, HBcrAg levels of seven cases and cccDNA levels of eight cases became negative. Subsequently, HBcrAg and cccDNA levels of five cases became positive again during the stable period. These dynamics implicated the re-infection of HBV in the graft liver.

Comparisons of the clinical features of HBcrAg and cccDNA levels. We divided patients into two groups according to their status of HBcrAg and cccDNA, and investigated their clinical features (Table 2). Positive group includes the patients with positive cccDNA or HBcrAg, negative group includes the patients with both negative.

In comparisons between the positive group and negative group, the number of patients being treated with entecavir was significantly lower in negative group ($P = 0.022$). Additionally, the stage of the graft liver was significantly lower ($P = 0.012$) in negative group. The grafts of the HBcrAg- and cccDNA-negative patients were in good condition in the lower fibrosis stages (median 0; range 0–1).

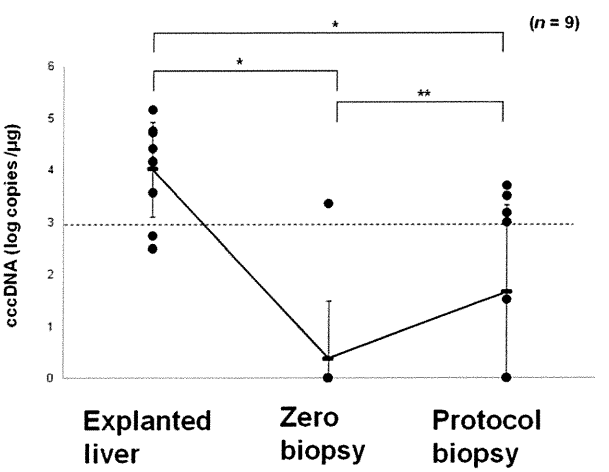

Figure 4 Serial changes of the covalently closed circular DNA (cccDNA) levels. cccDNA levels are represented as mean values; the closed circles show the values of the cccDNA levels in all phases. The error bars indicate standard deviations. The mean values of the cccDNA levels dropped during the time-zero biopsy but then gradually increased during the protocol biopsy (*$P < 0.001$ and **$P = 0.078$ indicate the significant differences between each period).

Discussion

In the present study, we demonstrated the usefulness of HBcrAg and cccDNA as markers of HBV after transplantation. As in our previous report,[12] we suggest that HBcrAg, which is a newly developed enzyme immunoassay,[9] is a possible method for detecting the dynamics of HBV after LT. However, HBcrAg consists of HBcAg, HBeAg, and p22cr, which is generated from cccDNA,

Table 2 Comparisons of the clinical features of HBcrAg and cccDNA levels

HBcrAg/cccDNA status	Positive group	Negative group	Positive versus negative
Patient M/F	10/2	7/1	NS
Day after transplantation†	854 (323–2163)	1674.5 (353–2456)	NS
Age†	55.5 (33–68)	56.5 (48–65)	NS
Serum HBV-DNA positive at LT (p/n)	7/5 (58.3%)	2/6 (33.3%)	NS
Serum HBeAg positive at LT (p/n)	1/11 (8.3%)	1/7 (14.3%)	NS
HBcAb-positive donor (p/n)	7/5 (58.3%)	1/7 (14.3%)	NS
Blood incompatibly (p/n)	1/11 (8.3%)	1/7 (14.3%)	NS
Presence of HCC at LT (p/n)	9/3 (75%)	7/1 (87.5%)	NS
RTI for prophylactic therapy after LT			
Use of LAM	3/12 (25%)	4/8 (50%)	NS
Use of ETV	9/12 (75%)	1/8 (12.5%)	$P = 0.022$
Use of ADV	0 (0%)	2/8 (25%)	NS
Use of LAM + ADV	0 (0%)	1/8 (12.5%)	NS
Immunosuppression after LT			
Use of TAC	10/12 (83.3%)	5/8 (62.5%)	NS
Use of CYA	0 (0%)	2/8 (25%)	NS
Use of MMF	2/12 (16.6%)	0 (0%)	NS
Use of TAC + MMF	0 (0%)	1/8 (12.5%)	NS
Liver function test			
Serum albumin (g/L)‡	39.2 (4.7)	40.0 (4.8)	NS
Child–Pugh score†	5.0 (5.0–9.0)	5.0 (5.0–6.0)	NS
Histology of LB			
Grade†	1.0 (0.0–3.0)	0.5 (0.0–1.0)	NS
Stage†	1.0 (0.0–3.0)	0.0 (0.0–1.0)	$P = 0.0027$
RAI score†	2.5 (0.0–5.0)	1.5 (0–4)	NS

Fisher's exact test for categorical variables.

†Mann–Whitney *U*-test for non-normally distributed variables, expressed as median (range).

‡Student's *t*-test for normally distributed variables, expressed as mean (SD).

ADV, adefovir; cccDNA, covalently closed circular DNA; CYA, cyclosporin A; ETV, entecavir; HBV, hepatitis B virus; HBcAb, hepatitis B core antibody; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B envelope antigen; HCC, hepatocellular carcinoma; LAM, lamivudine; LB, liver biopsy; LT, liver transplantation; MMF, mycophenolate mofetil; n, negative; NS, not significant; p, positive; RAI, Rejection Activity Index; RTI, reverse transcriptase inhibitor; SD, standard deviation; TAC, taclolimus.

and thus, it is questionable if HBcrAg truly reflects the viral pattern of HBV. Therefore, we designed this study to examine the usefulness of further analysis of cccDNA, which truly functions as a reservoir of HBV replication.

In the results of this study, a positive correlation between HBcrAg and cccDNA was shown, and this was consistent with a previous report on chronic hepatitis B.[11] These findings suggest the usefulness of monitoring HBV dynamics of patients after LTs because examinations of serum HBcrAg are less invasive methods compared with examinations of cccDNA levels in liver tissue. HBcrAg enables us to frequently check the HBV dynamics of patients, and it contributes to a reduction in the risk of HBV reactivation.[13]

However, as shown in Table 2, the results of the HBcrAg and cccDNA levels were not matched in 35% (7 of 20) of the patients. This may be due to a problem with the sensitivity of these two markers. We should use these markers cautiously because HBV might exist even if these were negative. Suzuki *et al.* reported that among the 13 patients with negative results for HBsAg, HBeAg, and HBV-DNA, all had positive results with cccDNA, while HBcrAg was positive in only seven patients.[11] In addition, cccDNA was also examined in a limited way because it was extracted from tissue from only a small part of the liver. Moreover, some reports have suggested that cccDNA can be detected in extrahepatic sites,[17] and thus, it is impossible to determine whether HBV exists with only one method. Therefore, we preferred to assess HBV dynamics with these two methods in order to overcome problems with sensitivity.

Interestingly, in the group with negative results for both of the two markers, the fibrosis stage was significantly lower compared with the other. This might reflect HBV activity after the LT. In addition, it was considered that keeping the two markers negative after LT may suggest the possibility of an extension of graft survival. But we observed only a limited period, further study of long-term outcome will be required.

The goal of this study was to determine the criteria for the appropriate prophylaxis of HBV related to LT with these two markers. Lenci *et al.* reported that 80.1% of the patients with undetectable intrahepatic cccDNA levels did not exhibit signs of HBV recurrence, even after withdrawal of the prophylaxis.[18] We thought that it might be possible to select patients more efficiently and correctly by using a method that combines examinations of HBcrAg and cccDNA. We observed one patient with both HBcrAg- and cccDNA-negative discontinued antiviral therapy.

Although the patient stopped antiviral therapy, he has not relapsed for 29 months (data not shown).

In conclusion, HBcrAg and cccDNA were helpful for the monitoring of HBV dynamics after LT and keeping a negative status of these markers might contribute to graft survival. In addition, using these methods, the criteria for the discontinuation of HBV prophylaxis could be clarified in the future.

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Review Article

Recent topics on α-fetoprotein

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Zinc-fingers and homeoboxes 2 (ZHX2) and zinc-finger and BTB domain containing 20 (ZBTB20) repress the postnatal expression of α-fetoprotein (AFP) by interacting with the AFP gene promoter regions. ZHX2 inhibits the expression of AFP and cyclins A and E. ZBTB20 is negatively regulated by CUX1, which promotes cell-cycle progression, suggesting that AFP reactivation is closely linked to hepatocyte proliferation. A slight elevation in the serum AFP level often occurs in patients with chronic hepatitis C in the absence of hepatocellular carcinoma (HCC) and is an independent risk factor for HCC development to complement the fibrosis stage. In addition, the sustained elevation of AFP after interferon therapy is a risk factor of HCC development. AFP levels are clinically useful in predicting the outcomes of liver transplantation and sorafenib therapy for HCC patients. A low preoperative AFP level is a predictor of long-term survival and is associated with a low recurrence rate of HCC after liver transplantation. AFP response (≥20% decrease in AFP during 6–8 weeks of treatment) rather than radiological outcomes is a significant prognostic factor for survival in sorafenib-treated HCC patients. Highly sensitive *Lens culinaris* agglutinin-reactive AFP (AFP-L3) is 5–10 times more sensitive than conventional AFP-L3, and useful for early detection of HCC in patients with total AFP below 20 ng/mL.

Key words: α-fetoprotein, chronic hepatitis C, hepatocellular carcinoma, highly sensitive *Lens culinaris* agglutinin-reactive α-fetoprotein, liver transplantation, sorafenib

INTRODUCTION

THE A-FETOPROTEIN (AFP) and albumin genes are similar in structure and tandemly arranged on the q arm of chromosome 4. Both genes are expressed at high levels in fetal liver. After birth, AFP expression decreases rapidly to an almost undetectable level, whereas albumin expression remains high.[1,2] The AFP gene is reactivated in pathological conditions such as hepatocellular carcinoma (HCC). The release of AFP gene repression in hepatocytes may be linked to hepatocarcinogenesis. The serum level of AFP is elevated in benign liver diseases, such as chronic viral hepatitis and liver cirrhosis without HCC.[3,4] Elevated AFP levels are linked to alanine aminotransferase elevation, hepatocyte regeneration and hepatic fibrosis.[3,4] A rising level of AFP over the first few hospital days indicates a better prognosis of acute liver failure.[4] In the present study, we reviewed the relationship between clinical features and serum AFP levels in patients with chronic hepatitis C (CHC) without HCC.

Assessment of AFP response after locoregional therapy for HCC, including surgical resection, radiofrequency ablation and transcatheter arterial chemoembolization, is simple and sensitive for detecting radiological tumor response, as well as an early objective screening tool for progression by imaging.[5,6] We reviewed the clinical usefulness of monitoring the serum AFP level during two newly established therapies for HCC, liver transplantation and administration of sorafenib. Finally, clinical usefulness of highly sensitive *Lens culinaris* agglutinin-reactive AFP (hs-AFP-L3) for detection and management of HCC is discussed.

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AFP GENE REGULATION

THE AFP GENE is positively regulated by transcription factors including HNF-1, HNF-3, HNF-4 and C/EBP that bind to specific elements in the promoter and enhancer regions.[7–9] These factors also bind to regulatory regions of the albumin gene,[10,11] which is consti-

tutively expressed in adult liver. Therefore, the existence of factors that specifically silence the AFP gene in adult liver has been supposed.[1,2,7–11] Indeed, two novel factors involved in postnatal AFP silencing have been identified, zinc-fingers and homeoboxes 2 (ZHX2)[12] and zinc-finger and BTB domain containing 20 (ZBTB20).[13]

In a study of the hereditary persistence of AFP in the liver of BALB/cJ mice, ZHX2 was identified as a postnatal repressor of AFP expression.[12] Shen *et al.* showed that ZHX2 overexpression decreases AFP secretion in human hepatoma cells expressing high AFP levels and that ZHX2 repression is governed by the human AFP promoter and requires intact HNF1 binding sites.[14] Hypermethylation of CpG islands in the ZHX2 promoter and the resultant loss of ZHX2 expression were detected in human HCC tissues, but not in surrounding non-tumor tissues.[15] These data suggest that ZHX2 contributes to human AFP repression in adult liver and may be involved in AFP reactivation in HCC. ZHX2 also represses glypican 3, an oncofetal gene.[16] Yue *et al.* reported that ZHX2 inhibits HCC cell proliferation by preventing the expression of cyclins A and E (Fig. 1a) and reduces the growth of xenograft tumors in mice.[17] Thus, they proposed that the loss of nuclear ZHX2 may be an early step in the development of HCC.

ZBTB20 is another repressor of AFP gene transcription in the liver. The main isoforms of ZBTB20 in humans and mice are 741 and 733 amino acids in length.[9] The liver-specific deletion of ZBTB20 resulted in the persistence of AFP expression in adult mouse liver.[13] ZBTB20 directly binds to a region of the AFP promoter between −108 and −53 and represses the AFP promoter activity.[13] Recently, it was shown that miR122, a liver-specific miRNA, indirectly modulates the expression of ZBTB20 and regulates AFP expression.[18] The miR122-silenced HCC cells exhibit a more invasive phenotype and produce more abundant AFP. In the miR122-silenced cells, the expression of CUX1, a transcription factor that regulates multiple processes including cell-cycle progression, is upregulated. CUX1 is a positive regulator of miR214. Because ZBTB20 is a target of miR214, the elevated expression of miR214 represses the ZBTB20 translation, followed by increased expression of AFP (Fig. 1b).[18]

Accordingly, ZHX2 inhibits the expression of both AFP and cyclins A and E.[17] ZBTB20 inhibits AFP expression and is regulated by CUX1, which promotes cell-cycle progression.[18] These findings suggest that AFP reactivation is closely linked to hepatocyte proliferation (Fig. 1).

AFP ELEVATION IN CHRONIC HEPATITIS C

MILDLY ELEVATED SERUM AFP levels are often seen in patients with CHC without HCC. The clinical significance of this mild elevation in serum AFP has been investigated. Hu *et al.* reported that in 357 patients with CHC without HCC, 82 (23.0%) patients had AFP levels of 10 ng/mL or more, and the AFP elevation was independently associated with stage III/IV hepatic fibrosis, the serum level of aspartate aminotransferase (AST) and prolonged prothrombin time.[3] The prevalence of elevated AFP (≥10 ng/mL) was 15.3% (28/183), 24.5% (25/102) and 42.0% (29/69) in stages 0–II, III and IV hepatic fibrosis, respectively.[3] In another report, elevated AFP levels (≥15 ng/mL) were observed in 23.9% (156/654) of CHC patients, and thrombocytopenia, AST elevation and AFP levels of 6 ng/mL or more were associated with advanced hepatic fibrosis.[4] Richardson *et al.* analyzed 258 275 AFP tests in a cohort

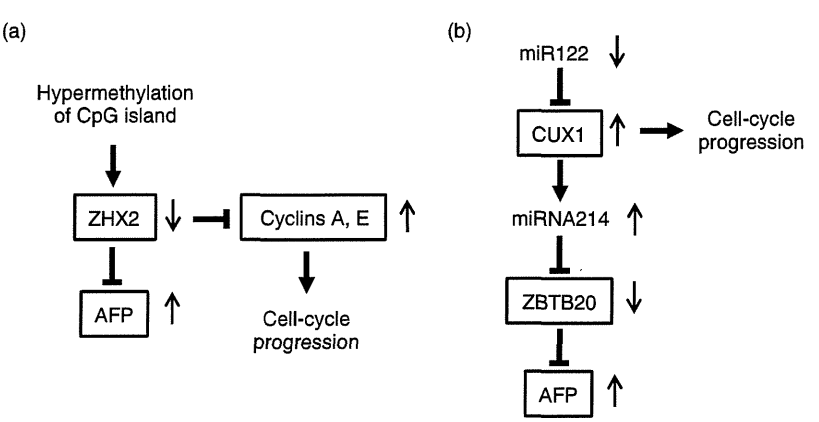

Figure 1 Schema of α-fetoprotein (AFP) gene regulation and cell-cycle control by zinc-fingers and homeoboxes 2 (ZHX2) (a) and CUX1/zinc-finger and BTB domain containing 20 (ZBTB20) (a).

of 76 347 hepatitis C virus (HCV)-infected patients.[19] Of these, 12 775 (16.6%) patients had cirrhosis, and 1488 (1.9%) patients developed HCC during the observation period. Among patients without HCC, significant determinants for increased levels of AFP included cirrhosis, high Model for End-Stage Liver Disease (MELD) score, and increased level of alanine aminotransferase.[19] Tateyama *et al.* reported that a slightly elevated AFP level is an independent risk factor for HCC to complement the fibrosis stage in a retrospective study of 707 CHC patients without HCC.[20] The 10-year cumulative incidence rates of HCC in patients with AFP levels of less than 6, 6–20 and 20 mg/mL or more at entry were 6.0%, 24.6% and 47.3%, respectively.[20]

In addition, the change in AFP level during interferon (IFN) therapy in CHC patients has been investigated. The serum level of AFP before pegylated (PEG) IFN/ribavirin (RBV) therapy predicts treatment outcome in CHC patients regardless of HCV genotype.[21] The serial AFP levels decreased after PEG IFN/RBV treatment, presenting in a time-dependent manner, specifically in patients who achieved a sustained virological response.[22] A decrease of serum AFP level after low-dose IFN therapy regardless of virological response has also been reported.[23–25] Moreover, Tamura *et al.* reported that increased serum AFP levels (≥10 ng/mL) at the end of IFN therapy was a significant variable affecting the development of HCC.[26] Osaki *et al.* also reported that among patients without a sustained virological response, a decrease in the AFP value (<10 mg/mL) by IFN therapy correlates with a reduced risk of HCC incidence after treatment.[27] Taken together, the results of these studies indicate that the sustained elevation of AFP (≥10 ng/mL) after IFN therapy is a risk factor of HCC development. In this regard, Akuta *et al.* reported that the substitution of amino acid 70 in the HCV core region of genotype 1b is an important predictor of elevated AFP in CHC patients without HCC.[28] The substitution of amino acid 70 in the HCV core region is related to non-sustained virological response by IFN therapy[29] and also to hepatocarcinogenesis.[30,31]

AFP LEVELS IN LIVER TRANSPLANTATION FOR HCC

THE SERUM LEVEL of AFP before liver transplantation for HCC is clinically significant. Several studies found that a low preoperative AFP level is a predictor of long-term survival and associated with a low recurrence rate of HCC after liver transplantation.[32–37] Mailey *et al.* analyzed 2253 patients who underwent orthotopic liver transplantation.[38] In this patient group, 1210 (53.7%), 805 (35.7%) and 238 (10.6%) patients had low (<20 ng/mL), medium (20–399 ng/mL) or high (≥400 ng/mL) AFP levels, respectively. The low AFP group had the greatest 4-year survival rate (76%) as compared to the medium (65%; $P < 0.001$) and high (57%; $P < 0.001$) AFP groups, and the improved survival in the low AFP group was still observed in patients with only stage II HCC.[38] Todo *et al.* analyzed a total of 653 patients with HCC who received living donor liver transplants in Japan.[39] In this study, the preoperative serum AFP levels were inversely correlated with patient survival: 83.8% at 1 year, 77.3% at 3 years and 72.2% at 5 years when AFP was less than 200 ng/mL ($n = 473$), and 64.9% at 1 year, 42.5% at 3 years and 34.0% at 5 years when AFP was 1000 ng/mL or more ($n = 48$).[39] Wang *et al.* reported 1-, 2- and 3-year recurrence-free survival rates of 83%, 63% and 53%, respectively, for patients with AFP levels of less than 20 ng/mL.[40] These survival rates were much greater than the corresponding rates for patients with AFP levels of 700 ng/mL or more (68%, 49% and 32% for the 1-, 2- and 3-year recurrence-free survival rates, respectively).[40] Fujiki *et al.* studied 144 HCC patients who received living donor liver transplants.[41] The 1-, 3- and 5-year recurrence-free survival rates for patients with AFP levels were less than 200 ng/mL in comparison to patients with AFP levels of 800 ng/mL or more were 97% versus 65%, 91% versus 40% and 90% versus 40%, respectively. However, the preoperative level of des-γ-carboxy prothrombin (DCP) (≥400 mAU/mL) was a stronger predictor of recurrence than the AFP level (≥800 ng/mL), and the DCP level (≥400 mAU/mL) was significantly related to microvascular invasion and poor differentiation of HCC cells.[41]

Pretransplant treatment of HCC patients with high AFP levels could be feasible and lead to similar intent-to-treat and post-transplant survival rates to those of patients with persistently low AFP levels.[42] Merani *et al.* analyzed 6871 HCC patients listed for liver transplantation and reported that patients with AFP levels decreased to 400 ng/mL or less by local pretransplant HCC treatment and patients with AFP levels persistently 400 ng/mL or less had similar dropout rates from the transplant list (10% for both groups) and similar post-transplant survival rates (89% vs 78% at 3 years, $P = 0.11$).[43] They concluded that only the last pretransplant AFP level independently predicted survival ($P < 0.001$), unlike the AFP level at the time of listing or AFP changes.[43] Toso *et al.* analyzed 5498 adult candidates for liver transplantation for HCC and 43 528 liver

transplant candidates with a non-HCC diagnosis.[44] They found that the dropout risk of HCC patients was predicted by the MELD score, HCC size, HCC number and AFP, and they calculated the dropout equivalent MELD (deMELD) points that express similar risks of dropout between HCC and non-HCC patients and allow for the management of both groups on a common waiting list.[44] The deMELD equation was obtained as follows:

$$\text{deMELD} = -25 + 0.1 \times \text{age} + 1.6 \times \text{MELD} + 1.6 \times \text{tumor size} + 1.3 \times \log(\text{AFP}),$$

+6 if tumor number ≥2, +0 if diagnosis = HCV, −1 if diagnosis = hepatitis B virus, +3 if diagnosis = alcohol, +3 if diagnosis = non-alcoholic steatohepatitis, +1 if diagnosis = hemochromatosis, +1 if diagnosis = other.

AFP LEVELS IN SORAFENIB-TREATED HCC PATIENTS

SORAFENIB IS AN antiangiogenic agent used to treat advanced HCC.[45,46] Sorafenib sometimes induces disappearance of contrast enhancement of HCC at the arterial phase, but rarely induces HCC shrinkage. Therefore, it is difficult to evaluate the antitumor effect of sorafenib or to predict its survival effect by imaging-based Response Evaluation Criteria in Solid Tumors (RECIST). Personeni *et al.* investigated the prognostic usefulness of a decrease in serum AFP levels and compared it to RECIST in 82 HCC patients treated with sorafenib.[47] AFP response (≥20% decrease in AFP during 8 weeks of treatment) rather than the radiological outcomes evaluated by RECIST was a significant prognostic factor for survival in multivariate analysis. The authors concluded that the assessment of AFP response was superior to RECIST in determining the response to sorafenib treatment.[47] Similarly, Yau *et al.* reported that decreased AFP levels (≥20% of the baseline level after 6 weeks of sorafenib) were significantly associated with progression-free survival both in 41 exploration patients and 53 validation patients.[48] When Kuzuya *et al.* evaluated the relationships between antitumor response based on imaging studies and early changes (2 and 4 weeks after starting sorafenib therapy) in AFP and DCP levels, they found that a significant early decrease in AFP levels was observed in the partial response and stable disease groups, while DCP levels increased despite therapeutic efficacy.[49] The authors speculated that the ischemic change of HCC cells may result in the elevation of DCP level, and concluded that AFP levels rather than DCP levels are useful for predicting antitumor responses during sorafenib therapy. In addition, the retrospective analysis of 66 patients treated with sorafenib revealed that assessment of overall survival by a change in AFP ratio of 1 or less at 8 weeks was better than that of more than 1 at 8 weeks ($P = 0.002$), but DCP ratio was not useful for assessment of overall survival.[50]

HS-AFP-L3

LENS CULINARIS AGGLUTININ-REACTIVE AFP, a fucosylated fraction of AFP, is a highly specific marker for HCC compared with AFP, and its elevation links to poor prognosis.[51] However, the advantage of AFP-L3 measured by conventional method had been limited due to its low sensitivity, especially in patients with total AFP below 20 ng/mL. To resolve this issue, a hs-AFP-L3 assay has been recently developed. There are several studies supporting the clinical utility of a newly developed hs-AFP-L3 assay in patients with low total AFP levels.[52–56] Toyoda *et al.* reported that sensitivity and specificity of hs-AFP-L3 for HCC in patients with total AFP below 20 ng/mL was 25–50% and more than 85%, respectively, at the cut-off level between 5% and 7%.[57] These results suggest that hs-AFP-L3 is 5–10-times more sensitive than conventional AFP-L3, maintaining high specificity. They proposed that hs-AFP-L3 elevation in patients with total AFP level below 20 ng/mL indicates poor prognosis of HCC and predicts detection of HCC in high-risk patients under surveillance. Thus it is possible that hs-AFP-L3 is a useful biomarker for detection and management of HCC, especially in patients with low total AFP.

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Laparoscopy-Assisted Hybrid Left-Side Donor Hepatectomy

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Abstract
Background Laparoscopic liver resection developed for live liver donors has the advantage of reducing the physical and mental stress in donors. However, its safety and efficacy still remain to be established. We aimed to evaluate the feasibility, safety and efficacy of laparoscopy-assisted hybrid donor hepatectomy (LADH) to obtain left side grafts.
Patients and methods A total of 31 consecutive live liver donors of left side liver grafts underwent LADH, including left lateral segmentectomy ($n = 17$) and left liver resection with or without the caudate lobe ($n = 14$) (LADH group). We compared the clinical data between the LADH group and the group of donors in whom traditional open donor hepatectomy was performed to procure the liver graft (open donor hepatectomy [ODH] group, $n = 79$).
Results Laparoscopy-assisted hybrid donor hepatectomy was feasible in all patients, and there was no mortality over a follow-up period of 13.9 ± 9.8 months. The operative time to procure a left-lobe graft was significantly longer in the LADH group (510 ± 90 min) than in the ODH group ($P < 0.001$). A large right lobe on CT (RPv distance) was identified as a significant risk factor for prolonged operative time ($P = 0.007$). Evaluation using the SF36-v2 questionnaire revealed faster recovery of the physical component summary score and bodily pain score in the LADH group than in the ODH group.
Conclusions Laparoscopy-assisted hybrid donor hepatectomy for procuring left side grafts was safe and effective up to the left liver with the caudate lobe. Left-lobe LADH in donors with a large right lobe should be carefully planned in view of the potential surgical difficulty.

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Introduction

In spite of the growing number of liver transplantations from brain-dead donors around the world, donor shortage still remains a significant problem. As a result, living donor liver transplantation (LDLT) is still necessary in Japan as well as other Asian and Western countries. Needless to say, the most important issue in LDLT is donor safety, and several reported donor deaths emphasize the great importance of this factor, and even minor morbidities should be minimized with the surgery conducted by an experienced surgeon [1, 2]. Donor surgery in live donors substantially affects quality of life, with the patients often developing wound infection, pain, and deformity [3–5]. A recent report of donor morbidities in Japan showed that the incidence of donor surgery-related morbidities was 8.4 % in total, and the leading morbidity was bile leak (2.6 %), followed by wound infection (1.2 %) [5].

Laparoscopy-assisted hybrid hepatectomy or laparoscopic liver resection has been developed for live liver graft donors, as well as for the treatment of benign or malignant tumors [3–6]. Several studies have shown its advantage over traditional open surgery in reducing the physical and emotional stress experienced by patients [7–13]. However, its safety and efficacy remain to be established.

Among the surgeries on live donors to procure liver grafts, that for obtaining a "left lobe including the caudate" graft is technically the most difficult, and very limited studies have reported the use of laparoscopic procedures to harvest left liver plus caudate lobe grafts [9]. Left liver plus

caudate lobe grafts have been used to obtain the maximum graft volume from the left side of the donor liver in adult-to-adult LDLT [14, 15]. It is important to adopt this principle, regardless of whether a laparoscopy-assisted procedure or traditional open surgery is employed.

We have experience in performing more than 140 live donor hepatectomies, as previously reported [16]. We also have sufficient experience in performing laparoscopy-assisted hepatectomy for the treatment of liver tumors. Based on this considerable experience, we began to perform laparoscopy-assisted hybrid donor hepatectomy, initially to obtain left lateral section grafts, and then, with accumulating experience, first, left liver without the caudate grafts, and finally left liver plus caudate lobe grafts.

The aim of the present study was to evaluate the safety and efficacy of laparoscopy-assisted hybrid donor hepatectomy (LADH) to procure left-side grafts, including left lateral section, left liver without the caudate, and left liver plus caudate lobe grafts.

Patients and methods

The study protocol was approved by the Human Ethics Review Committee of Osaka University Graduate School of Medicine (No. 750). A signed consent was obtained from each donor prior to operation. The study protocol was registered in the UMIN clinical trial registry (ID: UMIN000003886).

Study design

The study was a non-randomized prospective cohort study. The primary endpoint was mortality and morbidity of laparoscopy-assisted hybrid donor hepatectomy, and the secondary endpoint was the postoperative quality of life (QOL) of the living donors as evaluated in terms of analgesic requirement and the SF36v2 questionnaire for postoperative QOL.

Donor evaluation

Donor evaluation was based on the criteria approved by the ethics review committee of Osaka University. All living liver donors were adults between 20 and 65 years of age. Donor candidates with systemic diseases such as hypertension, diabetes mellitus, or psychiatric disease, and those receiving medications for any systemic disease were strictly excluded. Preoperative evaluation consisted of a complete history and physical examination, and laboratory tests (complete blood count, blood chemistry, coagulation profile, hepatitis B or C virus markers, and serological profiles for other infectious diseases). Donors also underwent chest and abdominal radiography, four-phase multidetector computed tomography (MD-CT) and drip-infusion cholangiography computed tomography (DIC-CT) with three-dimensional reconstruction. Liver volumetric analysis was conducted routinely with the Virtual Place software ver. 2.0 (AZE, Tokyo, Japan) and/or the Synapse Vincent 3D image analysis system (Fujifilm Corporation, Tokyo, Japan).

Graft selection

The criteria for donor selection have been described previously [16]. Briefly, the graft type was determined by the results of the volumetric study with MD-CT. The requirements for living donation were (1) an estimated volume of the remnant liver of more than 35 % of the whole liver volume of the donor, and (2) an estimated donor graft liver volume of more than 40 % of the recipient's standard liver volume (SLV).

Donor surgery

Open donor surgery

The methods employed for donor hepatectomies have been described previously [16]. All donors received a midline incision with bilateral subcostal incisions (Mercedes incision). Big incisions were an essential part of open donor surgery to secure the best possible field and assure donor safety during the operation. The bilateral costal incision was shorter in left lateral sectionectomy than in left lobectomy. Standard total length of incision was 25 cm in left lateral sectionectomy and 40 cm in left lobectomy. Surgery has been performed under general anesthesia without epidural anesthesia since July 2009. Basic techniques for donor hepatectomy were based on the strategy of no metal clips, no inflow occlusion, and minimal dissection of the liver hilum, as described previously [16].

Laparoscopy-assisted hybrid donor surgery (LADH)

A midline incision about 7 cm long was first made, and later extended an additional 1 cm or more, as needed. The round ligament and falciform ligament were divided. Liver wedge biopsy was obtained from segment 3 of the liver and sent for histopathological evaluation. A Gelport was placed and a 12 mm trocar was inserted through the Gelport, followed by establishment of pneumoperitoneum at 10 cm H_2O. A flexible 10 mm scope was used for the laparoscopic procedure. A 12 mm trocar was inserted at the umbilicus, and then the scope was reinserted from this second trocar, after which 5 mm trocars were placed as shown in Fig. 1, two for left lateral sectionectomy or three for left lobectomy. The left triangular ligament was

dissected up to the left hepatic vein under either full laparoscopic guidance or as a hand-assisted maneuver. For obtaining a left with caudate lobe graft, a 12 mm trocar was placed through the Gelport, and the caudate was mobilized from the inferior vena cava (IVC) under laparoscopic view (Fig. 1b). The short hepatic vein from the caudate was preserved if it measured more than 5 mm in diameter. The Arantius duct was transected, and the left and middle hepatic veins were mobilized from the IVC as far as possible. For left lobectomy with or without the caudate lobe, the right triangular ligament was dissected and the right lobe was mobilized with the hand-assisted laparoscopic surgery (HALS) technique. Dissection between the right adrenal gland and the liver was not necessary. Under the hybrid procedure, dissection around the right hepatic vein and pericaval region was carefully performed until the right lobe was fully mobilized. Pneumoperitoneum was ended after checking hemostasis. For left lobectomy, the incision was extended to 10–12 cm, then a retractor was placed. Dissection around the right hepatic vein was performed under direct vision at this point [17]. Cholecystectomy, hilar dissection, the liver hanging maneuver, and liver parenchymal dissection were performed under direct vision through the small midline incision in LADH. We applied the same procedure as that in the open technique in terms of not using any metal clips or inflow occlusion, with minimal dissection of the liver hilum.

Postoperative management and care

A drain was placed at the end of the operation, and was removed on postoperative day 2–3. Postoperative pain control was initiated immediately after operation with intravenous continuous fentanyl infusion at 0.5 μ/kg per hour for 40 h. Donors could receive bolus doses of fentanyl at 0.5 μ/kg per bolus every hour, as needed, up to 40 h after the operation, and flurbiprofen 50 mg or loxoprofen 50 mg thereafter.

Enhanced MDCT was performed on postoperative days (POD) 7, 14, and 28, and at 3, 6, and 12 months after operation. Doppler ultrasonography was performed on POD 1 to rule out the presence of a thrombus in the hepatic artery or portal vein. Donors were considered to be ready for discharge from the hospital on treatment with an oral proton pump inhibitor when the liver function tests were normal or improving satisfactorily, and they were capable of eating sufficient oral intake (more than 80 % of normal adult food).

Postoperative morbidities and evaluation of the health-related QOL after donor surgery

Postoperative morbidities were evaluated based on the Clavien–Dindo classification [18, 19]. Health-related QOL was evaluated preoperatively and at 1, 3, 6, and 12 months after the surgery with the Short Form-36, version 2 (SF36-v2) questionnaire [20].

Assessment of potential difficulty in left-lobe laparoscopy-assisted hybrid donor hepatectomy

Laparoscopy-assisted hybrid donor hepatectomy (LADH) could be more difficult to perform in obese or big male donors. Preoperatively, we calculated the distance between the abdominal wall and the front of the spine at the level of

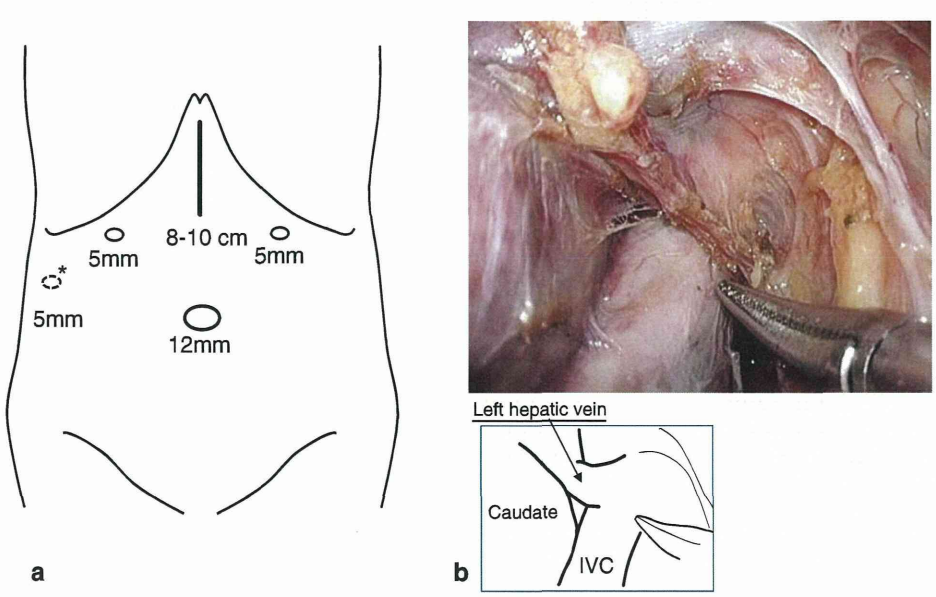

Fig. 1 Laparoscopy-assisted hybrid donor surgery. **a** Skin incision and trocar sites. An upper abdominal midline incision was made over a length of 7–8 cm for left lateral sectionectomy and a length of 10–12 cm for left lobectomy. A 12 mm trocar was placed through the umbilicus, and 2 trocars (5 mm) were placed in the hypochondriac region of either side. A third trocar (5 mm, *) was placed in the right flank for left lobectomy. **b** Mobilization of the left liver plus caudate. The Spiegel lobe of the caudate was completely mobilized under laparoscopic guidance

the portal bifurcation (WS distance), and the maximal distance between the surface of the right lobe and the portal vein bifurcation (RPv distance) on donor CT scans (Fig. 2a, b).

Evaluation of the feasibility and safety of laparoscopy-assisted hybrid donor liver surgery

Living donors who underwent LADH were divided into groups: those who underwent left lateral sectionectomy (LADH-lateral group) and those who underwent left lobectomy with or without the caudate lobe (LADH-left group). Living donors who underwent open donor hepatectomy were also divided into groups: those who underwent left lateral sectionectomy (open donor hepatectomy [ODH]-lateral group) and those who underwent left lobectomy with or without the caudate lobe (ODH-left group).

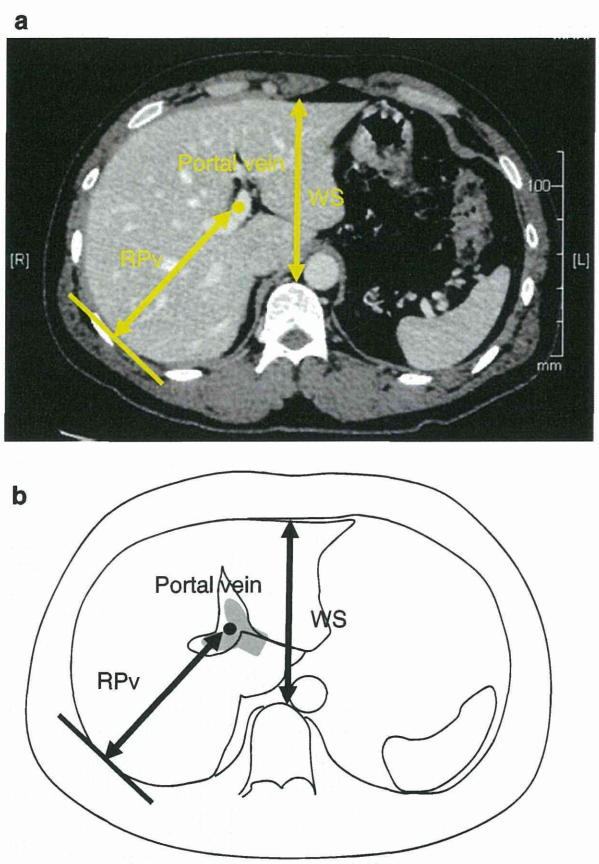

Fig. 2 Distance between the abdominal wall and the front of the spine at the level of the portal bifurcation (*WS distance*) and RPv distance. WS distance was defined as the distance between the abdominal wall and the front of the spine at the level of the portal bifurcation, and the maximal distance between the surface of the right lobe and the portal vein bifurcation (*RPv distance*) was defined as the maximal distance between the surface of the right lobe and the portal vein bifurcation on preoperative CT scans. **a** CT scan image. **b** Schematic view

The demographic characteristics, operative parameters, postoperative morbidities, results of the SF36-v2 questionnaire evaluation, analgesic requirement, and serum C-reactive protein levels measured preoperatively and on POD 1, 3, 7, and 14 were compared between the ODH and LADH groups.

The analgesic requirement was compared between the LADH ($n = 31$) and recent open donor groups (after July 2009 [$n = 21$]), when we stopped using epidural anesthesia and started to use intravenous fentanyl for 40 h after surgery in July 2009.

Statistical analysis

Results are expressed as mean ± standard deviation. Statistical examination of the correlations was based on the Pearson product-moment correlation. Clinical data of the donors were compared with Student's t test. P values <0.05 were considered to indicate statistical significance.

Results

A total of 31 consecutive live liver donors of left-side liver grafts underwent LADH between April 2009 and March 2012; of these, 17 donors underwent left lateral sectionectomy (LADH-lateral group), including one case of in situ S3 monosegmentectomy, and 14 donors underwent left lobe resection with or without the caudate lobe (LADH-left group). We compared the clinical outcomes between the LADH group ($n = 31$) and donors who had undergone open donor hepatectomy (ODH group; $n = 79$) prior to this period in our hospital, which were either open left lateral sectionectomy (ODH-lateral group; $n = 32$), including one case of reduced-left lateral sectionectomy or open left lobe resection with or without the caudate lobe (ODH-left group; $n = 47$).

There was no perioperative or postoperative mortality in any of the donor groups, and all the donors were healthy without any sustained physical or mental problems at 13.9 ± 9.8 months after the donor hepatectomy.

The demographic characteristics of the donors were similar between the LADH group and the ODH group (Table 1). The length of the midline incision was 7.5 ± 0.7 cm in the LADH-lateral group and 10.5 ± 1.4 cm in the LADH-left group. The operative time was 375 ± 65 min in the LADH-lateral group and 508 ± 94 min in the LADH-left group; the operative time was significantly longer in the LADH-left group than in the ODH-left group ($P < 0.001$). The volume of blood loss was similar between the LADH and ODH groups. The

postoperative length of hospital stay was 9.0 ± 2.3 days in the LADH-lateral group and 11.5 ± 3.6 days in the LADH-left group, which were significantly shorter than those for the donors who had undergone open surgery ($P = 0.019$).

The operative time was similar between the donors who underwent left lobe resection with the caudate ($n = 6$) or without the caudate lobe ($n = 8$), and it was not associated with the body mass index (BMI) or the WS distance. Of note, the operative time increased as the RPv distance increased ($P = 0.014$, $r = 0.637$) (Fig. 3a, b). The operative time was significantly longer in the donors with an RPv distance equal to >10 cm ($n = 6$) as compared with donors with an RPv distance of less than 10 cm ($n = 8$) ($P = 0.007$) (Fig. 3c). No significant correlation was observed between the volume of blood loss and the RPv distance or WS distance.

Laparoscopy-assisted hybrid donor hepatectomy was feasible, without any need for conversion to open surgery, in all patients in the LADH group. During the laparoscopic procedure, two incidental injuries (one to the diaphragm and one to the right hepatic vein) occurring during mobilization of the right lobe were successfully managed by finger compression under the HALS technique and subsequent suturing under direct vision through the midline incision. In one of the patients, however, elongation of the midline incision to 15 cm was necessitated; in the other, the procedure was completed through the planned 12 cm midline incision.

Table 1 Characteristics of the laparoscopy-assisted hybrid donor hepatectomy (LADH) group and the open donor hepatectomy (ODH) group

	LADH ($n = 31$)	ODH ($n = 79$)	P value
Age, years	35.8 ± 8.4	37.8 ± 10.1	0.369
Gender (male)	13 (41.9 %)	54 (68.4 %)	0.011
Body mass index (BMI), kg/m^2	21.3 ± 3.6	22.6 ± 3.1	0.075
Type of resection			
Left lateral section (LLS)	16	31	0.174
Reduced left lateral section (rLLS)	1	1	(Left vs. LLS)
Left lobe without caudate (left)	8	10	
Left lobe with caudate (left-C)	6	37	
Operative time, min	435 ± 103	383 ± 73	0.005
Estimated blood loss, ml	353 ± 396	456 ± 347	0.197
Length of hospital stay after surgery, days	10.3 ± 3.3	18.3 ± 16.7	0.019
Complication (Clavien–Dindo grade)			
1	1 (3.2 %)	7 (8.9 %)	0.653
2	0	1 (1.3 %)	
3a	2 (6.5 %)	8 (10.1 %)	
3b	0	1 (1.3 %)	
4/5	0	0	

After the donor surgery the amount of pain medication needed up until the seventh POD after 40 h of systemic fentanyl infusion was compared between the LADH group ($n = 31$) and the recent ODH group ($n = 21$), and was found to be similar between the two groups (Fig. 4a). Likewise, the serum C-reactive protein (CRP) levels after surgery were similar between the LADH and recent ODH group (Fig. 4b).

Postoperative morbidity, defined with the Clavien–Dindo classification [18], was established as grade ≥2 in two donors (6.7 %) with delayed gastric emptying which required fiberoptic endoscopy ($n = 2$) for correcting rotation of the stomach, and both recovered within 2 weeks after the donor surgery. No bile leak or other morbidity was observed.

There was no mortality related to the LADH procedure among the graft recipients. The graft survival rate of the 17 pediatric recipients who received the left lateral section grafts from the LADH-lateral group was similar to that of the 32 pediatric recipients who received the left lateral section grafts from the ODH-lateral group ($P = 0.877$, log rank test) (Fig. 5a). The graft survival rate of the 14 recipients (9 adults and 5 children) who received the left lobe grafts in the LADH-left group was slightly better but statistically similar to that of the 47 recipients (32 adults and 15 children) who received the left lobe grafts in the ODH-left group ($P = 0.237$, log rank test) (Fig. 5b).

A total of 29 donors from the LADH group could be evaluated by the SF36-v2 questionnaire. Comparison with the preoperative test results revealed that the scores for all six components decreased significantly at 1 month after the surgery; thereafter, the physical functioning (PF) score, general health perception (GH) score, vitality (VT) score, social functioning (SF) score, and mental health (MH) score recovered by 3 months, while the role physical (RP) score, bodily pain (BP) score, and role emotional (RE) score recovered by 6 months after the surgery. The PCS score, which was decreased at 1 month after the surgery, recovered by 6 months, and the mental component summary (MCS) score, which was decreased at 1 month after the surgery, recovered by 3 months (Fig. 6).

Discussion

Despite close attention being paid to preventing donor mortality and morbidity in living donor hepatectomies, it is inevitable to encounter them at a certain incidence.

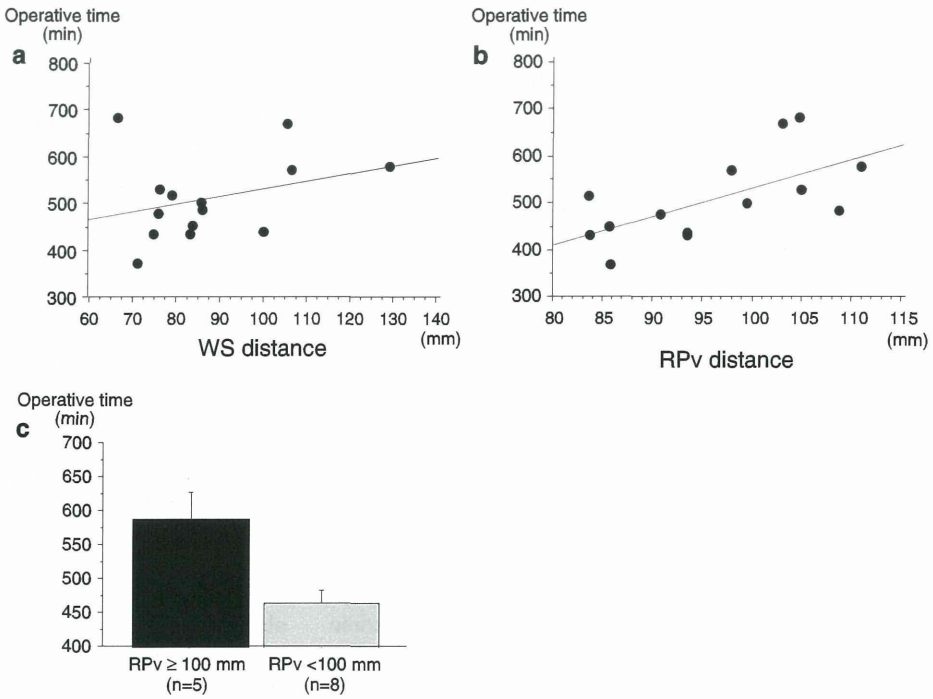

Fig. 3 Relationship between the WS distance and RPv distance and the operative time in the LADH-left group ($n = 14$). **a** WS distance and operative time. There was no significant correlation between these two parameters. ($r = 0.239$). **b** RPv distance and operative time. There was a significant correlation between the RPv distance and the operative time; operative time (min) $= -76.4 + 6.10 \times$ RPv (mm); $P = 0.014$, $r = 0.637$. **c** The operative time was significantly longer in donors with an RPv distance equal to or >10 cm ($n = 6$) than in those with an RPv distance of <10 cm ($n = 8$) ($P = 0.007$)

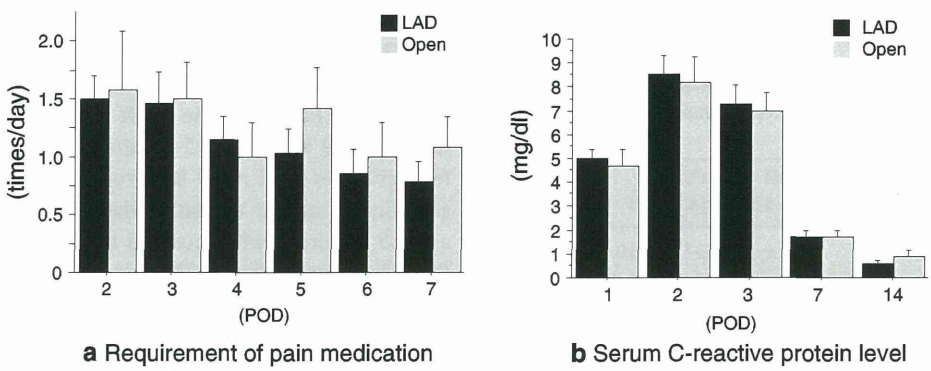

Fig. 4 Analgesic agent requirement and serum C-reactive protein level. (LADH: $n = 31$, ODH group: $n = 21$). **a** Analgesic agent requirement from postoperative day (POD) 2 to POD 7. While the requirement was higher in the ODH group after POD 5, there was no significant difference between the LADH and ODH groups. **b** The serum CRP level peaked on POD 2 in both groups, with no significant difference in the level change between the LADH and ODH groups

Therefore many surgeons consider that the traditional open donor hepatectomy with a big incision is appropriate, merely for reasons of safety. In addition, donor protection is very important in terms of reduction of physical and mental stresses, and also provision of support for recovery from the surgery to a healthy daily life as before the operation. Laparoscopic surgery was introduced in the field of donor hepatectomy, first from left lateral sectionectomy [6] and on to right lobectomy [17], and these techniques have been rapidly spread worldwide. However, parenchymal dissection in laparoscopic view is not always a familiar technique to most hepatobiliary surgeons who are experts in open donor hepatectomies. LADH has been developed based on its advantageous characteristics of less invasiveness for living liver donors and the familiarity of direct parenchymal dissection to hepatobiliary surgeons. One of the other important features of this procedure is the safety we have observed during laparoscopic surgery because of the advantages of hand-assisted surgery.

In our series there were two significant complications during right lobe mobilization: a right diaphragmatic injury and an injury to the right hepatic vein. In each case the surgeon was able to make a successful recovery, initially using fingers in the hand-assisted technique, without any

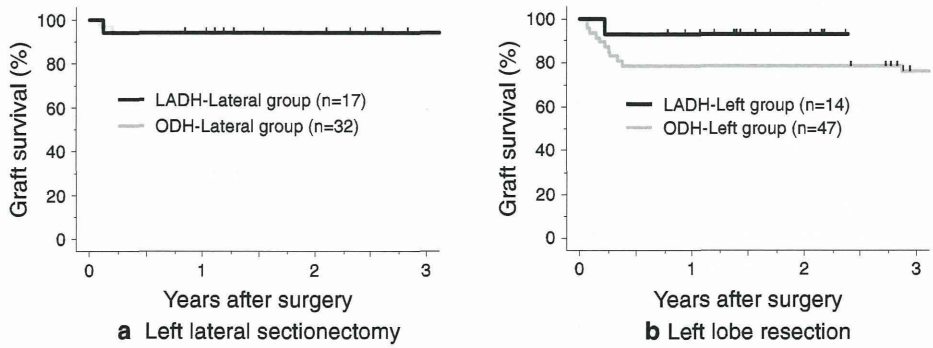

Fig. 5 The graft survival rates after liver transplantation. **a** Left lateral sectionectomy. The graft survival rates were similar between the LADH and the ODH groups ($P = 0.877$, log rank test). **b** Left lobe resection with caudate or without caudate. The graft survival rate in the LADH group was slightly better than that of the ODH group, although there was no significant difference between the LADH and the ODH groups ($P = 0.237$, log rank test)

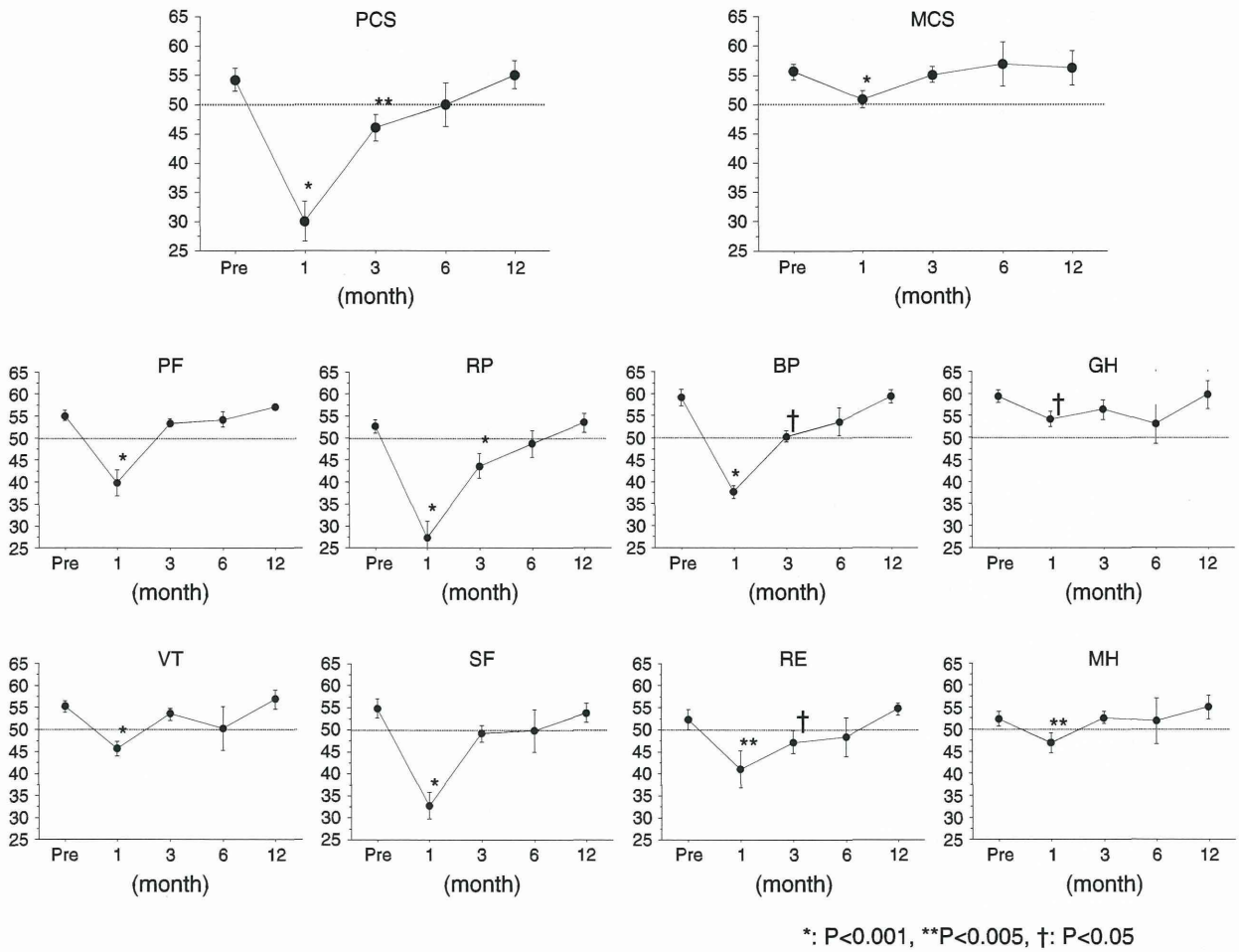

Fig. 6 Evaluation by the Short Form-36, version 2 (SF36-v2) questionnaire. *PCS* physical component summary, *MCS* mental component summary, *PF* physical functioning, *RP* role physical, *BP* bodily pain, *GH* general health perceptions, *VT* vitality, *SF* social functioning, *RE* role emotional, *MH* mental health. * $P < 0.001$; ** $P < 0.005$; † $P < 0.05$

problem under HALS technique. Nevertheless, the safety and efficacy of LADH has not been established, and few feasibility studies are reported [7, 9–11]. Therefore, the purpose of the present study was to investigate the safety and efficacy of the laparoscopic procedure for procuring left liver grafts.

The technique of LADH is quite demanding, and adequate experience with both open donor hepatectomy and laparoscopic mobilization of the left and right hemi-liver is required. Thus, it is important to ensure that LADH is performed by surgeons with adequate experience in both donor hepatectomy and laparoscopic liver mobilization, under the assumption that "experienced" surgeons in donor hepatectomy would be able to perform donor left lobectomy with the caudate by themselves without any supervision.

We have reported the adequacy of our open donor hepatectomy previously, and have also performed laparoscopic hepatectomies very actively. Having established these two bases, we started to perform LADH in a stepwise manner, from LADH-lateral to LADH-left surgery; we believe that this stepwise approach was fundamental from the point of view of preserving the donor safety. We conducted research to determine the best sites for ports, the number of ports, the method for dissecting the liver hilum and hepatic veins in 10 cases of laparoscopy-assisted hybrid left lateral sectionectomy, and then proceeded to left lobe surgery with or without the caudate.

The target length of the midline incision was 7–8 cm for LADH-lateral in our series. This was sufficient to perform hilar dissection and dissection of the liver parenchyma for lateral segmentectomy. For left lobectomy, the incision was extended to 10 cm or longer to ensure an adequate view of the liver parenchyma for dissection. Thus, the mean length of the midline incision was 7.5 ± 0.7 cm for left lateral sectionectomy and 10.5 ± 1.4 cm for left lobectomy. It is noteworthy that the length of the skin incision was uniform in spite of differences in body constitution or BMI in LADH, which could not be expected in open donor hepatectomy.

In our series blood loss was similar between the LADH and ODH groups. The operative time for left lateral sectionectomy was similar between the LADH and ODH groups, but that for left lobectomy was much longer in the LADH group than in the ODH group ($P < 0.001$). No improvement was seen even with case experience (data not shown), suggesting that the longer operative time for left lobectomy was needed because of the small incision in the LADH group.

The operative time in the LADH-left group was associated with the RPv distance, but not with the WS distance. An RPv distance of over 10 cm was identified as a significant risk factor for a prolonged operative time. At first, in fact, we hypothesized that the WS distance might influence the difficulty level of left-lobe LADH. However, no correlation was noted between the WS distance and the duration of operation. We then calculated the RPv distance, because we thought that the difficult cases tended to have a larger right lobe. During the left-lobe LADH procedure, the right lobe is mobilized and rotated toward the midline incision to allow performance of hybrid surgery through the small midline incision. Our results showed that the longer the RPv distance, the longer the duration of left-lobe LADH, suggesting that the volume of the right lobe of the liver had a greater impact on this procedure than the depth of the abdomen. Because left-lobe LADH is expected to be more difficult and to take a longer time in donors with an RPv distance >10 cm in left-lobe LADH, the operation type and explanations to the donors should be carefully conducted preoperatively.

Again in our series, two incidental events occurred during LADH that may have been avoided by a surgeon with greater experience in laparoscopic right lobe mobilization. However, both incidental injuries were easily treated with the help of a hand inserted into the abdomen, which is the one of the advantages of the HALS technique. In case of unexpected incidents such as these, the HALS technique is quite useful and safer than pure laparoscopic surgery, which is one of the reasons why we adopted HALS. It is fundamental in donor surgery not to expose the donor to any avoidable danger.

Postoperative morbidity was rather rare in the LADH group, and the length of hospital stay after surgery was shorter in the LADH group than that in the ODH group ($P = 0.028$), indicating that the safety of the procedure was comparable to that of the well-established open procedure. Serum CRP level is one of the markers of acute-phase reactions to surgery; however, in the present series it failed to reflect any advantage of the laparoscopic procedure, with the smaller skin incision, over the open procedure. In studies comparing open and laparoscopic colorectal surgery, no significant differences in the serum levels of interleukin (IL)-1, IL-6, IL-8, or interferon γ (IFN-γ), all of which are known to be acute-phase cytokines, were found between the laparoscopic surgery and open surgery groups [21]. These results showed that the invasiveness of the surgery was not different between the open and laparoscopic techniques, at least as evaluated by measurements of the serum cytokine levels, even though the patients in the LADH group recovered more rapidly after surgery and discharge than those of the ODH group.

The length of hospital stay after surgery was significantly reduced in the LADH group. Although the length of hospital stay was much longer as compared with that reported from the West in both the open and LADH groups [7], it is our policy to keep the donors in the hospital until the absence of any influence of the surgery in the daily lives of the patients, except for requirement of a minimal amount of pain medications.

In the short-term evaluation, the analgesic requirement during the first week after surgery was similar between the LADH and ODH groups. However, in the longer-term evaluation, the QOL after surgery as evaluated using the SF36-v2