introduced minimum dissection of the bile duct at the hilus to preserve the blood supply to the bile duct. The GRWR was reduced from 0.8% to 0.6% as a new graft selection criterion; LDLT using an LL graft subsequently increased in our institution.

This study aimed to evaluate the usefulness of this new surgical procedure and to report on the donor morbidity with RL and LL liver transplantation by comparing the postoperative liver function and complication details between the two groups.

Patients and methods

Donors and grafts

Between April 2006 and March 2012, 429 consecutive LTs [411 LDLT, 16 deceased donor liver transplantations (DDLTs) and two domino LTs] were performed at Kyoto University Hospital. Of the 411 living donors, 168 underwent a donor operation for an RL graft with (n = 11) or without (n = 157) the middle hepatic vein (MHV), and 140 underwent a donor operation for an LL graft with (n = 76) or without (n = 64) the caudate lobe. In this study, donors of the lateral segment, extended lateral segment, mono segment and posterior segment were excluded from the analysis. The donor and graft demographic data, duration of surgery, intraoperative blood loss, postoperative hospital stay, liver function test and complications/morbidity were evaluated. As a postoperative liver function test, serial changes in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil) and prothrombin time–international normalized ratio (PT-INR) were measured in the peripheral blood on postoperative days (PODs) 1, 2, 3, 5, 7, 10, 14, 21 and 28.

This study was approved by the Ethics Committee of Kyoto University and was conducted in accordance with the Declaration of Helsinki of 2000.

Donor and graft selection

Potential donors underwent blood tests, including blood counts, blood chemistry, infection analyses, tumour markers, blood type determination, human leucocyte antigen typing and mixed lymphocyte reaction assays. Nonalcoholic steatohepatitis (NASH) was evaluated using the homoeostatic model assessment index. For all potential donors, multidetector-row computed tomography (CT) imaging was performed to detect hepatic anatomical variations and to evaluate the donor's whole liver volume, graft volume and remaining donor liver volume. Instead of a needle biopsy of the donor liver, the liver-to-spleen CT attenuation value ratio (L/S ratio) was used in our institution to assess steatosis of the liver. The L/S ratio indicates the grade of hepatic steatosis. The optimal L/S ratio to predict more than 30% hepatic steatosis is 1.1 [9]. Since November 2002, HepaVision2 (MeVis, Bremen, Germany), which is software specifically developed for image analysis and risk analysis of the liver, was used to estimate the graft volume and congestive volume in the graft [10]. Using raw data obtained from multislice CT, various anatomic sites can be visualized, and volumetry of the portal and venous regions can be performed. Conventional volumetry, including whole liver volume, RL volume with or without MHV, LL volume and remnant liver volume, was calculated. To assess the biliary tree, routine magnetic resonance cholangiopancreatography (MRCP) was performed.

Our graft selection criteria were modified in April 2006; the GRWR minimum was reduced from 0.8% to 0.6%, and LL became the first choice for the graft whenever feasible. The RL was considered when the GRWR of the LL was <0.6%. However, if the remnant liver volume was <30% of the total liver volume, the person was excluded as a donor candidate.

Surgical procedure for the donor operation

The donor operation was performed as described previously [11,12]. Briefly, under general anaesthesia, a thorough laparotomy was performed. After a retrograde cholecystectomy, a catheter was inserted into the cystic duct for intraoperative cholangiography. Depending on the type of liver graft donation, the right or left portal vein and the right or left hepatic artery were isolated, and the demarcation line was noted by temporally clamping the graft's side vessels. Liver parenchymal transection was performed using the Cavitron Ultrasonic Surgical Aspirator (CUSA system, Valleylab Inc., Boulder, CO, USA) and bipolar electrocautery without inflow occlusion prior to cutting the hepatic duct. The cutting line of the hepatic duct was carefully determined based on intraoperative cholangiography using a static X-ray film unit. After parenchymal transection was initiated, hilar dissection was performed without dissection of the pericholedochal tissue to preserve the blood supply around the hepatic duct. The hepatic duct within the hilar plate was separated with fine scissors, and the stumps of the remnant hepatic duct were meticulously closed with 6–0 polydioxanone absorbable monofilament sutures. To ensure the absence of bile leakage and stricture, a cholangiogram was performed again. Systemic administration of heparin was performed following complete parenchymal transection. Thereafter, the hepatic artery, portal vein and hepatic vein were cut sharply. All the grafts were perfused ex situ via the portal vein with a histidine-tryptophan-ketoglutarate solution (Custodiol; Chemie GmbH, Alsbach-Hahnlein, Germany).

Modifications of the surgical procedure in the donor operation

We modified the surgical procedure in the donor surgery as follows. Since April 2002, a biliary decompression tube has been placed through the cystic duct into the residual bile duct to prevent bile leakage from the bile duct stump in donors with difficulty in hepatic duct end closure. Since June 2004, abdominal drainage has been reduced to bile duct drainage only, except in donors at high risk for biliary complications based on the intraoperative findings. Since April 2006, parenchymal transection has been started before cutting the hepatic duct, and we have introduced the method of hilar dissection during parenchymal transection. This procedure minimizes the dissection of the bile duct, thus preserving the blood supply to the bile duct of both the graft and the remnant liver.

Definition of the grade of postoperative donor complications

Postoperative donor complications were graded according to the Clavien classification [13]. Complications worse than Grade IIIa were recognized as major complications. Hyperbilirubinemia was defined as serum total bilirubin levels >3 mg/dl at POD 7 without coagulopathy.

Statistical analysis

All the values are presented as the means and standard deviations for each group. Categorical variables were compared with the chi-square test or Fisher's exact test. The statistical analyses of the groups at each time point were tested with 2-way analysis of variance and Bonferroni's *post hoc* test. For the patient survival analysis, the Kaplan–Meier method with the log-rank test was used. P values <0.05 were considered statistically significant. The analysis was performed using GRAPHPAD PRISM software version 5 (GraphPad Software, La Jolla, CA, USA).

Results

Changes in graft types

Figure 1 demonstrates the changes in the numbers of LDLT graft types since April 2006. After the introduction of the new modified graft selection criteria, LDLT using LL grafts gradually increased. Since 2009, the frequency of LL grafts has become nearly equal to that of RL grafts.

Donor demographics

The demographic data of the RL and LL donors are summarized in Table 1a. Regarding the donor and graft characteristics, there was no significant difference in the gender distribution or donor age between the RL and LL donors. The mean body weight and body mass index of the LL donors were significantly higher than those of the RL donors. Regarding the donor operative outcomes, the duration of surgery and blood loss were comparable between the RL and LL donors. We administered no homologous blood transfusions to donors of either graft type. No significant difference was found in the postoperative hospital stay between the RL and LL donors.

Postoperative liver function test

There were no significant differences in the peak serum AST or ALT levels between the RL (324 ± 193 IU/l and 325 ± 161 IU/l, respectively) and LL (289 ± 136 IU/l and 339 ± 150 IU/l, respectively) donors. However, the peak serum T-Bil level was significantly higher in the RL donors (4.3 ± 1.8 mg/dl) than in the LL donors (2.6 ± 2.1 mg/dl) ($P < 0.05$).

Figure 2a and b show the postoperative serial changes in the serum T-Bil and PT-INR levels, respectively. The RL donors presented a significant increase in serum T-Bil during the week after donor surgery ($P < 0.05$) (Fig. 2a). Moreover, the PT-INR was significantly higher in the RL donors at PODs 1, 2, 3 and 5 than that in the LL donors ($P < 0.05$) (Fig. 2b). Essentially, liver damage persisted longer in the RL donors than in the LL donors.

Donor complications

No donor mortality or life-threatening complications were observed in the 308 living donor hepatectomies during this study period. The donor complications are shown in Table 2. The overall complication rate of the RL donors (59.5%) was significantly higher than that of the LL donors (30.7%) ($P < 0.001$). The rates of biliary complications in the RL and LL donors were 7.1% and 5.0%, respectively (P = NS). Regarding the complications in the RL donors, there were 12 biliary complications, including 11 instances of bile leakage (6.5%) and one biliary stricture (0.6%). The number of bile leakage and biliary stricture occurrences in the LL donors was 5 (3.6%) and 2 (1.4%), respectively. Regarding the rate of each biliary complication, there were no significant differences between the donors. The mean time to the occurrence of bile leakage in the 11 RL and 5 LL donors was 10.7 and 12.0 days, respectively. One biliary stricture in an RL donor occurred 33 days after donor surgery. Two LL donors were diagnosed as having biliary strictures 47 and 118 days after surgery.

Regarding nonbiliary complications, intra-abdominal fluid collection had the highest incidence in the RL donors, occurring in 26 (15.5%) donors and significantly more often than in the LL donors (2.1%) ($P < 0.001$). Moreover,

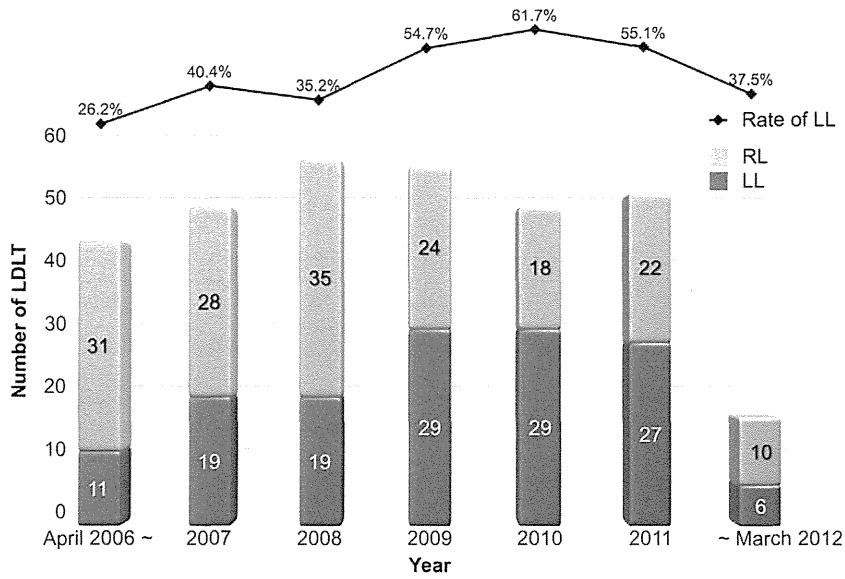

Figure 1 Changes in the numbers of graft types. Modified graft selection criteria were introduced in 2006; the use of LL grafts for LDLT has increased compared with RL grafts.

Table 1. (a) Donor and graft demographic data. (b) Comparison of recipients by graft type.

Variables	RL (n = 168)	LL (n = 140)	P value
(a)			
Gender (M/F)	75/93	50/90	0.130
Age (year)	43.9 ± 12.6	42.8 ± 11.4	0.452
Body weight (kg)	58.7 ± 10.5	63.7 ± 11.2	<0.001*
BMI (%)	21.9 ± 2.8	22.8 ± 2.9	0.009*
Actual graft volume (g)	667 ± 106	417 ± 85	<0.001*
GRWR (%)	1.02 ± 0.21	0.87 ± 0.25	<0.001*
Duration of operation (min)	406 ± 82	420 ± 77	0.135
Blood loss (g)	345 ± 224	338 ± 257	0.799
Hospital stay (day)	17.7 ± 29.4	14.6 ± 7.0	0.196
(b)			
Gender (M/F)	155/53	40/100	<0.0001*
Recipient age (year)	50.8 ± 12.9	44.1 ± 18.5	0.0003*
Recipient body weight (kg)	67.2 ± 11.5	50.2 ± 11.4	<0.0001*
Recipient MELD score	17.6 ± 7.5	19.0 ± 8.9	0.2524

*P < 0.05.

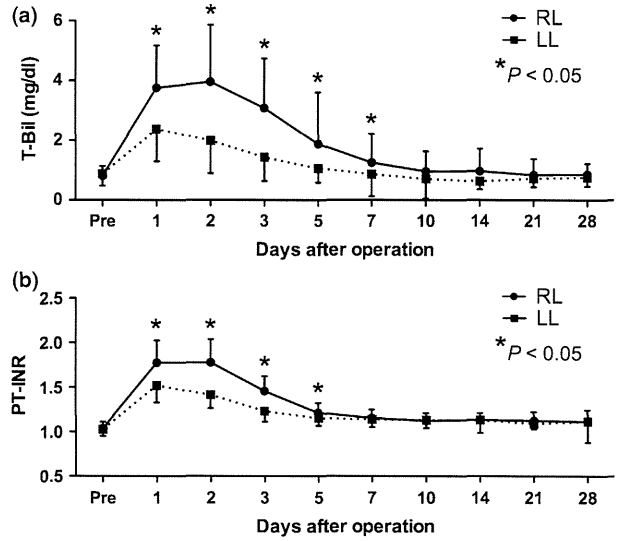

Figure 2 Postoperative serial changes in serum T-Bil and PT-INR levels. (a) The serum T-Bil levels of the RL graft donors were significantly higher than those of the LL graft donors 1 week after donor surgery (P < 0.05). (b) PT-INR levels were significantly higher in the RL graft donors at PODs 1, 2, 3 and 5 than in the LL graft donors (P < 0.05).

the rate of hyperbilirubinemia was notably higher in the RL donors (2.4%) than in the LL donors (0.7%); however, this difference was not significant. The highest incidence of complications in the LL donors involved skin wound problems, which occurred more frequently in the LL donors (11.4%) than in the RL donors (6.0%); however, this difference was not significant. No significant differences were found in other abdominal complications. Two venous thromboses, including one hepatic venous thrombosis and one portal venous thrombosis, occurred in the RL donors. These two donors with venous thromboses were diagnosed via postoperative CT scan, and the thrombi were detected in the MHV and the stump of the right portal vein, respectively. These thrombi disappeared after anticoagulant therapy, and these two donors were discharged without any further complications.

Regarding extra-abdominal complications, pleural effusion occurred significantly more frequently in the RL donors (5.4%) than in the LL donors (0.7%) (P < 0.05).

Table 2. Comparison of donor complications.

	RL (n = 168)	LL (n = 140)	P value
All complications	100 (59.5%)	43 (30.7%)	<0.001*
Biliary complications	12 (7.1)	7 (5.0)	0.484
Bile leakage	11 (6.5)	5 (3.6)	0.307
Biliary stricture	1 (0.6)	2 (1.4)	0.593
Other abdominal complications			
Fluid collection	26 (15.5)	3 (2.1)	<0.001*
Skin wound problem	10 (6.0)	16 (11.4)	0.101
Small bowel obstruction	1 (0.6)	2 (1.4)	0.593
Intra-abdominal abscess	2 (1.2)	–	0.503
Drug-induced hepatotoxicity	4 (2.4)	7 (5.0)	0.360
Massive ascites	3 (1.8)	–	0.254
Hyperamylasemia	3 (1.8)	1 (0.7)	0.629
Hyperbilirubinemia	7 (4.2)	1 (0.7)	0.076
Gastritis/intractable ulcer	1 (0.6)	–	1.000
Venous thrombosis	2 (1.2)	–	0.503
Extra-abdominal complications			
Pleural effusion	9 (5.4)	1 (0.7)	0.025*
Atelectasis	1 (0.6)	1 (0.7)	1.000
Pneumothorax	1 (0.6)	–	1.000
Pulmonary embolism	–	1 (0.7)	0.455
Fever of unknown origin	5 (3.0)	1 (0.7)	0.226
Others	13 (7.7)	2 (1.4)	0.014*

*P < 0.05.

There were no significant differences in other extra-abdominal complications.

Postoperative complication grade

The postoperative complication grades of the RL and LL donors are shown in Table 3. In the RL donors, the complication rates of Grades I and II were 22.0% and 23.2%, respectively. Regarding major complications, the incidence of Grade IIIa complications was 14.3%; no complications worse than Grade IIIb occurred during this study period. The 24 Grade IIIa complications included nine biliary complications, six cases of intra-abdominal fluid collection, two skin wound problems, two intra-abdominal abscesses, four pleural effusions and one pneumothorax. Of the 11 RL donors with bile leakage, endoscopic nasobiliary drainage was necessary in 3 (1.8%) donors (Grade II), and percutaneous drainage of the bile was performed in 8 (4.8%) donors (Grade IIIa). Moreover, endoscopic retrograde biliary drainage was necessary for 1 (0.6%) biliary stricture (Grade IIIa).

The complication rates of Grades I, II and IIIa were 13.6%, 8.6% and 7.9%, respectively, in the LL donors. Regarding major complications, 11 Grade IIIa complications (six biliary complications, three skin wound problems, one small bowel obstruction and one pulmonary embolism) occurred. A Grade IIIb complication occurred in only 1 (0.7%) LL donor. In this donor, reoperation (hepaticojejunostomy) was necessary for delayed biliary stricture 7 months after donor surgery.

Table 3. Complication grades of RL and LL donors.

	I	II	IIIa	IIIb
All complications				
RL	37 (22.0%)	39 (23.2%)	24 (14.3%)	–
LL	19 (13.6%)	12 (8.6%)	11 (7.9%)	1 (0.7%)
Biliary complications				
RL	–	3 (1.8%)	9 (5.4%)	–
LL	–	–	6 (4.3%)	1 (0.7%)

	Complication rate	P
Severe complications above grade III		
RL	14.3% (24/168 cases)	0.15
LL	8.6% (12/140 cases)	

The complication rate of RL donors was Grade IIIa and was comparable with that of LL donors (P = 0.15). During this study period, no Grade IV or V complications were experienced.

Comparison of biliary complications in RL donors during different periods

Table 4 shows the biliary complication rate and major complication rate in the RL donors according to different periods. The biliary complication rate in the RL donors decreased from 14.2% (Period 1: June 1990 to March 2002) to 12.9% (Period 2: April 2002 to March 2006). During this study period (Period 3: April 2006 to March 2012), the overall biliary complication rate of RL donors was 7.1%, which was significantly lower than that during Period 1. The major complication rate showed a tendency to decrease over time, but there were no significant differences among the three periods.

Comparison of recipients by graft type

The recipients' characteristics and the model for end-stage liver disease (MELD) score are summarized in Table 1b. Significant differences were found in recipient gender distribution, age and body weight. The MELD scores of the

Table 4. Biliary complications in RL donors according to period.

Period	Biliary complication rate (%)	P	Major complication rate (%)
June 1990 to March 2002	14.2		16.6
April 2002 to March 2006	12.9	0.03	17.8
April 2006 to March 2012	7.1		14.3

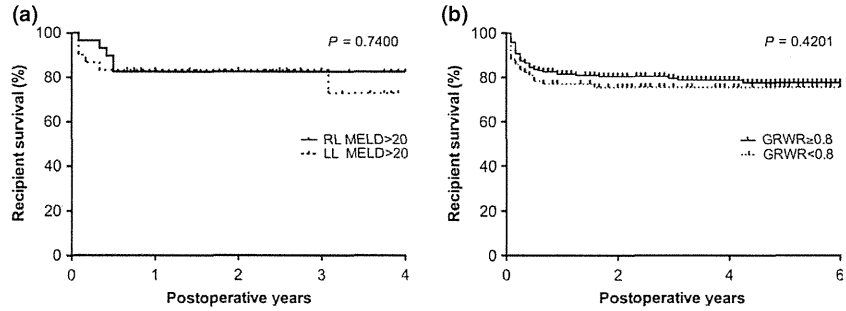

Figure 3 Comparison of recipient survival according to MELD score and GRWR. (a) Survival rate of recipients with MELD scores >20 was comparable between RL and LL graft recipients. (b) The log-rank test found no statistically significant differences in survival rate of recipients with GRWR ≥0.8 and those with GRWR <0.8.

RL and LL recipients were 17.6 ± 7.5 and 19.0 ± 8.9, respectively. Figure 3a shows that the survival rate of the LL recipients with MELD scores >20 was comparable with that of the RL recipients (P = 0.7400). Moreover, as shown in Fig. 3b, there was no significant difference in the survival rate between recipients with a GRWR <0.8% and those with a GRWR ≥0.8% (P = 0.4201).

Discussion

In LDLT, the safety of the donor is the ultimate priority. However, we experienced the first instance of donor death in Japan in 2003, which resulted from liver failure caused by RL graft donation and NASH. Trotter reported 13 living liver donor deaths that were 'definitely' related to donor surgery [14]. Similarly, Ringe identified 33 living liver donor deaths, including 21 deaths related to the surgical procedure. Of these 21 deaths, at least 14 cases involved RL graft donation. They concluded that the incidence of donor death was 0.1–0.3% and likely reached 0.5% when using an RL graft for adult-to-adult LDLT [15]. Therefore, the selection of graft type is very important for donor safety.

We previously reported on the surgery-related morbidity in LDLT, in which multivariate analysis demonstrated that RL donation was an independent risk factor for complications in these donors [8]. Recently, the feasibility and usefulness of LL grafts for adult-to-adult LDLT have been reported [16,17]. The safety of LL grafts for adult-to-adult LDLT was compared with that of RL grafts. Moreover, the outcomes of LL grafts in LDLT were not inferior to those of RL grafts in LDLT. However, small-for-size syndrome (SFSS) occurred more often in LL graft LDLT than in RL graft LDLT.

There are still many debates regarding the relationship between the MELD score and post-transplant outcomes. Theoretically, a high MELD score is associated with poor patient and graft survival following LT. Hayashi reported that there was no correlation between the 1-year survival rate and the MELD score [18]. Although Li reported that MELD score emerged as an independent risk factor for SFSS, they also reported that the 1- and 3-year survival and postoperative complication rates were similar between recipients with high MELD scores and those with low MELD scores [19,20]. RL grafts are recommended for recipients with MELD scores >20 [21], yet the present study showed that LL grafts were feasible for recipients with MELD scores >20, with a survival rate comparable with that of RL grafts (Fig. 3a). In addition, our recent study indicated that pretransplant sarcopenia and the absence of perioperative nutritional therapy were independent risk factors for post-transplant mortality in patients undergoing LDLT, whereas the MELD score is not [22]. However, the recipient's pretransplant general condition (MELD, portal hypertension, renal dysfunction, pretransplant diabetes mellitus, etc.) is a risk factor affecting recipient and graft survival [21,23,24]. Thus, we may modify our graft selection criteria in the future.

We modified our graft selection criteria and introduced new recipient portal pressure control in 2006. The lower limit for GRWR was reduced to 0.6%. Regarding the recipient's portal pressure, our previous study showed that a final portal pressure <15 mmHg was an important factor for better outcomes in adult-to-adult LDLT using smaller grafts [25]. The present study demonstrated that the recipient survival rate was comparable between patients with a GRWR <0.8% and those with a GRWR ≥0.8% (Fig. 3b). Therefore, we believe that small grafts can be safely available via portal pressure control without SFSS, and LDLT using an LL graft has been increasingly used in our institution since 2006 (Fig. 1).

Our study showed that the overall complication rate of RL donors was significantly higher than that of LL donors and that Clavien grade II and IIIa complications occurred significantly more frequently in RL donors than in LL donors. Additionally, LL donors showed significantly better improvement in serum T-Bil and PT-INR levels. Our study demonstrated that LL donors could achieve earlier liver function recovery after donor hepatectomy than RL

donors. This result was due to a sufficient remnant liver volume. Hyperbilirubinemia and coagulopathy persisted in RL donors. Because the early recovery of postoperative liver function can contribute to donor safety, LL grafts offer significant advantages in donor safety compared with RL grafts. Essentially, LL grafts offer significant advantages in postoperative liver regeneration. Previously, we had primarily performed RL graft donor surgeries in LDLT, and we reported several studies on donor morbidity in RL and LL grafts [8,26,27]. Based on our experience in donor surgery, our donor surgery procedure and postoperative management have been continuously modified and improved in the effort to reduce severe donor morbidity. The team experience of each organ transplant centre is believed to be the most critical factor in reducing donor morbidity; living donor morbidity after liver donation has been strongly correlated with the experience of the centre [28,29].

Biliary complications remain among the most common problems associated with LDLT, and the rate of these complications has been reported to range from 0% to 38.6% [30]. Table 5 shows the biliary complications and compares the RL donors with the LL donors; our previous reports are included [8,16,26,31–35]. Nearly all centres have reported that the overall biliary complication rate was higher in the RL donors than in the LL donors. Anatomic variations in the biliary tract might significantly contribute to the higher biliary complication rate in the RL donors. The anterior and posterior segmental branches of the right hepatic duct (RHD) often diverge immediately proximal to the bifurcation of the RHD and left hepatic duct (LHD). Therefore, the RHD must be cut within a few millimetres of the bifurcation. Furthermore, RL grafts often have multiple biliary orifices, whereas LL grafts usually have a single orifice. RL grafts also have larger biliary stamps than LL grafts, which result in a higher incidence of biliary leakage among RL donors.

The hilar plexus is a set of communicating arcade vessels that bridge the right and left hepatic arterial systems, and it is located within the hilar plate. The blood supply to the RHD arises from both the right hepatic artery and the hilar plexus. The LHD is supplied by a plexus that is continuous with the plexus at the confluence of the RHD and the common bile duct. Therefore, dissection of the hilar plate and hepatic artery can easily destroy the communicating arcade of the hilar bile duct. Minimizing the dissection of the hepatic artery and portal vein is important to avoid damage to the arterial plexus and to ensure that the surrounding tissues remain attached to the common and branched hepatic ducts. The high hilar dissection technique during recipient hepatectomy might contribute to reducing the biliary complications by preserving adequate blood supply to the bile duct [36]. We have applied this hilar dissection technique in donor hepatectomy since April 2006.

According to a previous study from our institution, the biliary complication rate in RL donors decreased from 18.6% [26] to 14.5% [27]. In 2010, Iida updated our published experiences and reported that the incidence of biliary complications in RL donors from April 2002 to March 2006 decreased to 12.9% [8]. During this study period (April 2006 to March 2012), the overall biliary complication rate of RL donors was 7.1%, and we did not experience complications worse than Clavien grade IIIb in the RL donors. We believe that surgical refinements and innovations, especially in the dissection of the bile duct, have assisted in reducing the incidence of biliary complications.

Although the biliary complication rate of the LL donors was lower than that of the RL donors, the Clavien grade IIIb complication of biliary stricture occurred in only one LL donor. This previously reported donor had a trifurcated portal vein and a rare biliary anomaly [37]. When rare biliary anatomy is observed in the LL, precise preoperative identification of the biliary anomalies is essential.

Table 5. World reports of biliary complications in living donors for liver transplantation.

First author (reference)	Year	Institute	Number of donor (RL:LL)	Number of biliary complication		Biliary complication rate (%) (RL:LL)
				Bile leakage (RL:LL)	Biliary stricture (RL:LL)	
Fujita [23]	2000	Kyoto, Japan	43:99	8:3	N/A	18.6:3.0
Lo [28]	2003	Multicenter, Asia	561:334	34:8	6:0	7.1:2.4
Hwang [29]	2006	Seoul, Korea	591:571 (*89)	3:2	5:0	1.4:0.4†
Shio [30]	2008	Kyoto, Japan	434:297 (*237)	43:5	9:3	11.1:2.4†
Taketomi [16]	2009	Fukuoka, Japan	69:137	3:2	4:2	10.1:2.9
Iida [8]	2010	Kyoto, Japan	500:762 (*493)	53:36	8:2	12.2:4.9†
Kousoulas [31]	2010	Hanover, Germany	36:51 (*47)	1:3	N/A	2.8:5.9†
Shin [32]	2012	Seoul, Korea	698:129 (*108)	N/A	N/A	2.0:0.9†
Present study	2014	Kyoto, Japan	168:140	11:5	1:2	7.1:5.0

N/A, not applicable.

*The number of lateral segment.

†The number of lateral segment graft.

Therefore, we routinely perform preoperative MRCP and intraoperative cholangiography with a static X-ray film unit in all live donors to prevent biliary complications. We should undertake continuous efforts to improve the surgical technique in an effort to reduce biliary complications.

In conclusion, our study demonstrated superior recovery of postoperative liver function and lower morbidity in LL donors compared with RL donors. Moreover, the survival rate of the LL recipients was comparable with that of the RL recipients, even in high-risk recipients with MELD scores >20. The biliary complication rate has gradually decreased due to surgical innovations regarding hilar dissection. To reduce morbidity in living donors, further surgical technique refinements and careful postoperative management are necessary. An LL graft is recommended as the first choice in LDLT, given adequate portal pressure modulation.

Authorship

JI: Participated in analysing the data and writing the paper. TI, MM, TU, SY, TH, KO, YF, AM and TK: Participated in collecting the data and performing the surgery. SU: Participated in creating the research design and performing the surgery.

Funding

The authors have declared no funding.

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ORIGINAL ARTICLE

Impact of Quality as Well as Quantity of Skeletal Muscle on Outcomes After Liver Transplantation

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Intramuscular fat accumulation has come to be associated with loss of muscle strength and function, one of the components of sarcopenia. However, the impact of preoperative quality of skeletal muscle on outcomes after living donor liver transplantation (LDLT) is unclear. The present study evaluated the intramuscular adipose tissue content (IMAC) and psoas muscle mass index (PMI) in 200 adult patients undergoing LDLT at our institution between January 2008 and October 2013. Correlations of IMAC with other factors, overall survival rates in patients classified according to IMAC or PMI, and risk factors for poor survival after LDLT were analyzed. IMAC was significantly correlated with age ($r = 0.229$, $P = 0.03$) and PMI ($r = -0.236$, $P = 0.02$) in males and with age ($r = 0.349$, $P < 0.001$) and branched-chain amino acid (BCAA)-to-tyrosine ratio ($r = -0.250$, $P = 0.01$) in females. The overall survival rates in patients with high IMAC or low PMI were significantly lower than those for patients with normal IMAC or PMI ($P < 0.001$, $P < 0.001$, respectively). Multivariate analysis showed that high IMAC [odds ratio (OR) = 3.898, 95% confidence interval (CI) = 2.025-7.757, $P < 0.001$] and low PMI (OR = 3.635, 95% CI = 1.896-7.174, $P < 0.001$) were independent risk factors for death after LDLT. In conclusion, high IMAC and low PMI were closely involved with posttransplant mortality. Preoperative quality and quantity of skeletal muscle could be incorporated into new selection criteria for LDLT. Perioperative nutritional therapy and rehabilitation could be important for good outcomes after LDLT. *Liver Transpl 20:1413-1419, 2014.* © 2014 AASLD.

Received May 18, 2014; accepted July 23, 2014.

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Sarcopenia is defined as a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength, with a risk of adverse outcomes such as physical disability, poor quality of life, and death.[1] Recent evidence has shown that sarcopenia is an independent predictor of lower disease-free and overall survival in various kinds of diseases.[2-4] In patients with liver cirrhosis (LC), protein malnutrition, which is caused by decreased protein synthesis and disturbed energy metabolism, can cause a decrease in skeletal muscle mass. In recent studies, sarcopenia was found to be present in approximately one-third of patients with hepatocellular carcinoma (HCC) and LC who were being evaluated for liver transplantation (LT), and sarcopenia was found to be an independent prognostic factor for overall and recurrence-

Abbreviations: AUC, area under the curve; BIA, bioelectrical impedance analysis; BCAA, branched-chain amino acid; BTR, BCAA-to-tyrosine ratio; CI, confidence interval; CT, computed tomography; DXA, dual energy X-ray absorptiometry; GRWR, graft-to-recipient weight ratio; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IMAC, intramuscular adipose tissue content; IMAT, intramuscular adipose tissue; LC, liver cirrhosis; LDLT, living donor liver transplantation; LT, liver transplantation; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis; OR, odds ratio; OS, overall survival; PBC, primary biliary cirrhosis; PMI, psoas muscle mass index; PSC, primary sclerosing cholangitis; ROC, receiver operating characteristic; ROI, region of interest; SD, standard deviation; TPMT, transversal psoas muscle thickness.

Potential conflict of interest: Nothing to report.

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DOI 10.1002/lt.23970
View this article online at wileyonlinelibrary.com.
LIVER TRANSPLANTATION.DOI 10.1002/lt. *Published on behalf of the American Association for the Study of Liver Diseases*

free survival in patients with HCC after partial hepatectomy.[5,6] In the field of LT, Englesbe et al.[7] reported that central sarcopenia, evaluated by the size of the psoas muscle as measured by computed tomography (CT) scan, strongly correlated with post-LT mortality. We have shown that pretransplant low skeletal muscle mass and low body cell mass measured by bioelectrical impedance analysis (BIA) were independent risk factors for death after living donor liver transplantation (LDLT).[8]

The European Working Group on Sarcopenia in Older People has recommended that the definition of sarcopenia include not only low muscle mass but also low muscle function.[1] Recently, the increase of intramuscular adipose tissue (IMAT) with aging has been identified as a potential contributor to declining strength and quality of muscle.[9] Loss of muscle strength is acknowledged to depend on both decrease in muscle mass and accumulation of intramuscular adipose tissue. Kitajima et al.[10,11] evaluated skeletal muscle steatosis by measuring intramuscular adipose tissue content (IMAC) and found that skeletal muscle steatosis was linked to the pathogenesis and severity of nonalcoholic steatohepatitis (NASH). However, the impact of IMAC on survival in patients undergoing LDLT is unclear. The present study evaluates the quality as well as quantity of skeletal muscle by measuring IMAC and psoas muscle mass index (PMI), respectively, on preoperative CT. We investigated the impact of IMAC and PMI on outcomes in patients undergoing LDLT.

PATIENTS AND METHODS

Patients

There were 235 adult (age ≥18 years) patients who underwent LDLT at Kyoto University Hospital between January 2008 and October 2013. Thirty-five patients who did not undergo preoperative plain CT imaging at the umbilical level were excluded from this study. Therefore, in total 200 patients (95 men, 105 women) were enrolled in the study. The study was approved by the Ethics Committee of Kyoto University and was conducted in accordance with the Declaration of Helsinki of 1996.

The median patient age was 54 years (range = 18-69 years). Sixty patients were ABO incompatible, and 140 were identical or compatible. The median Model for End-Stage Liver Disease (MELD) score was 18 (range = 5-55). The Child-Pugh classifications were C, B, and A for 125, 60, and 15 patients, respectively. The indications for LDLT were HCC (n = 67), hepatitis B virus (HBV)- or hepatitis C virus (HCV)-associated LC (n = 38), progressive intrahepatic cholestatic diseases including primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC; n = 34), biliary atresia (n = 19), acute liver failure with unknown etiology (n = 9), alcoholic LC (n = 6), metabolic liver diseases (n = 6), Budd-Chiari syndrome (n = 4), and other causes (n = 17). Orthotopic LDLT was performed with a left lobe graft for 93 patients, a right lobe graft for 102 patients, a posterior segment graft for 4 patients, and a whole liver graft as a domino LT from a patient with familial amyloid polyneuropathy for 1 patient. The median graft-to-recipient weight ratio (GRWR) was 0.89 (range = 0.54-1.46). The selection criteria for the recipients, the surgical techniques for the donor and recipient, and the immunosuppressive regimen have been described previously.[12-14]

Image Analysis

IMAC was calculated as previously described by Kitajima et al.[11]: IMAC = region of interest (ROI) of the multifidus muscle (Hounsfield units)/ROI of subcutaneous fat (Hounsfield units). In our image analysis, we used plain CT images on admission, usually 7 to 14 days before transplantation. Subfascial muscular tissue in the multifidus muscle on the preoperative plain CT cross-sectional image at the umbilical level was precisely traced, and CT values (in Hounsfield units) were measured with the Aquarius NET server (TeraRecon, San Mateo, CA; Fig. 1A). CT values were measured for ROIs of 4 circles on subcutaneous fat away from major vessels (Fig. 1B). The mean values of these 4 ROIs were used as the ROI of subcutaneous fat.

The cross-sectional areas of the right and left psoas muscles were measured by manual tracing from preoperative CT images at the same level (Fig. 1C). PMI was calculated by normalizing the cross-sectional areas for height (cm^2/m^2).

Cutoff Values of IMAC and PMI

To select the optimal cutoff values of IMAC and PMI that classify the poor prognostic group after LDLT, receiver operating characteristic (ROC) curves were calculated. The cutoff values were selected on the basis of best accuracy in relation to an outcome (death). The cutoff values of IMAC in males and females were −0.375 [area under the curve (AUC) = 0.689, $P = 0.005$] and −0.216 (AUC = 0.693, $P = 0.002$), respectively. The cutoff values of PMI in males and females were 6.868 (AUC = 0.621, $P = 0.07$) and 4.117 (AUC = 0.688, $P = 0.003$), respectively.

Analyzed Parameters

The correlations of IMAC or PMI with other factors, such as patient age, sex, MELD score, Child-Pugh classification, total lymphocyte count, prealbumin, zinc, branched-chain amino acid (BCAA)-to-tyrosine ratio (BTR), ammonia, and skeletal muscle mass measured by BIA were analyzed. The overall survival rate after LDLT was investigated in patients classified according to IMAC or PMI. The prognostic factors were analyzed on the basis of the following variables: age of recipient, age of donor, sex, original disease, ABO compatibility, MELD score, Child-Pugh classification, graft type (right or left), GRWR,

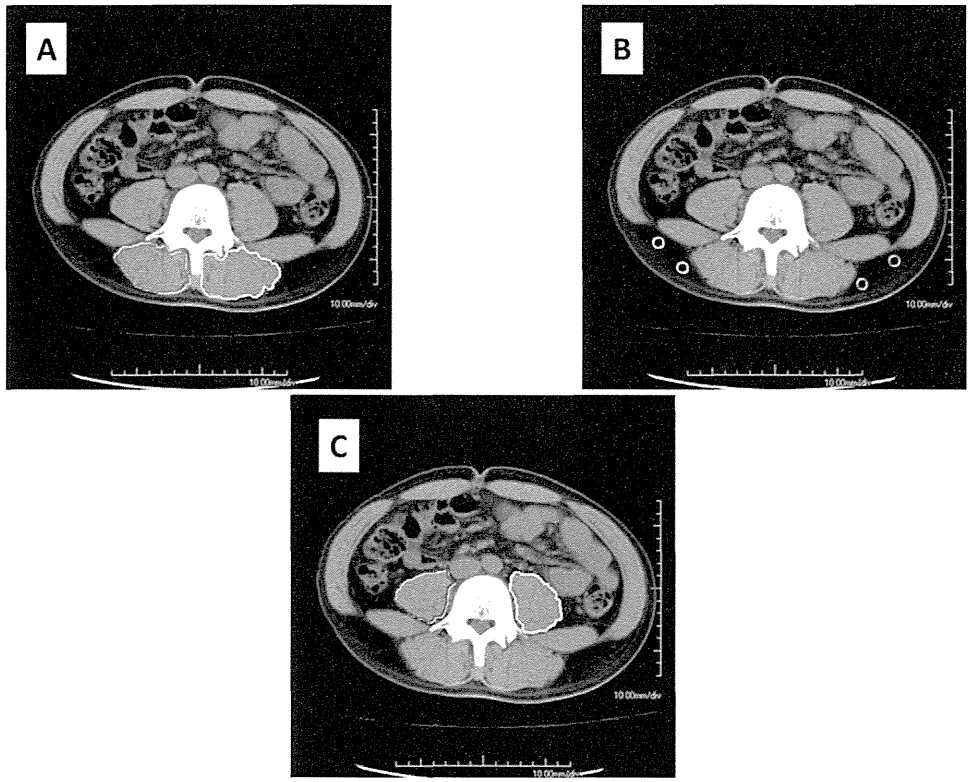

Figure 1. Cross-sectional CT images at the umbilical level. (A) Subfascial muscular tissue in the multifidus muscle was precisely traced. (B) Four small circles were placed on subcutaneous fat away from major vessels. ROIs of the multifidus muscle and the subcutaneous fat (Hounsfield units) were measured with the Aquarius NET server. (C) The areas of bilateral psoas muscle were measured by manual tracing.

duration of surgery, estimated blood loss, and preoperative IMAC and PMI.

Statistical Analysis

Data are presented as mean ± standard deviation (SD) for continuous variables. Continuous variables were compared by χ^2 test or Fisher's exact test when appropriate. Any variable identified as significant ($P < 0.05$) or with $P < 0.10$ in univariate analysis with the above-mentioned tests was considered a candidate for multivariate analysis with multiple logistic regression models. Cumulative overall survival rates were calculated by Kaplan-Meier methods, and differences between curves were evaluated with the log-rank test or the Mantel-Cox test. $P < 0.05$ was considered significant. All statistical data were generated in JMP 11 (SAS Institute, Cary, NC) and Prism 6 (GraphPad Software, La Jolla, CA).

RESULTS

Correlations of Preoperative IMAC With Other Factors

For males, a significant positive relationship was observed between IMAC and patient age ($r = 0.229$, $P = 0.03$; Fig. 2A), and a significant negative relationship was observed between IMAC and PMI ($r = -0.236$, $P = 0.02$; Fig. 2B). For females, a significant positive relationship was observed between IMAC and age ($r = 0.349$, $P < 0.001$; Fig. 2C), and a significant negative relationship was observed between IMAC and BTR ($r = -0.250$, $P = 0.01$; Fig. 2D).

Overall Survival Rate After LDLT

The overall survival rate after LDLT was significantly lower in patients with high IMAC (n = 90) than in patients with normal/low IMAC (n = 110; $P < 0.001$; Fig. 3A). The median survival times for patients with high IMAC and normal IMAC were 21.9 (range = 0.2-67.6) and 32.4 (range = 0.5-70.2) months, respectively. The overall survival rate also was significantly lower in patients with low PMI (n = 88) than in patients with normal/high PMI (n = 112; $P < 0.001$; Fig. 3B). The median survival times for patients with low PMI and normal PMI were 17.6 (range = 0.2-69.7) and 33.9 (range = 0.5-70.2) months, respectively.

A total of 55 patients died in this follow-up period. Forty-nine of fifty-five patients (89.1%) died within the first year after LDLT, and the other 6 patients (10.9%) died after the first year after LDLT. The causes of death for 39 patients with high IMAC were as follows: sepsis (n = 18); pulmonary complications (n = 5); graft failure including antibody-mediated rejection and chronic rejection (n = 5), cerebral bleeding (n = 6); and others (n = 5). The causes of death for 16 patients with normal IMAC were as follows: sepsis (n = 7); pulmonary complications (n = 2); graft failure (n = 4); cerebral

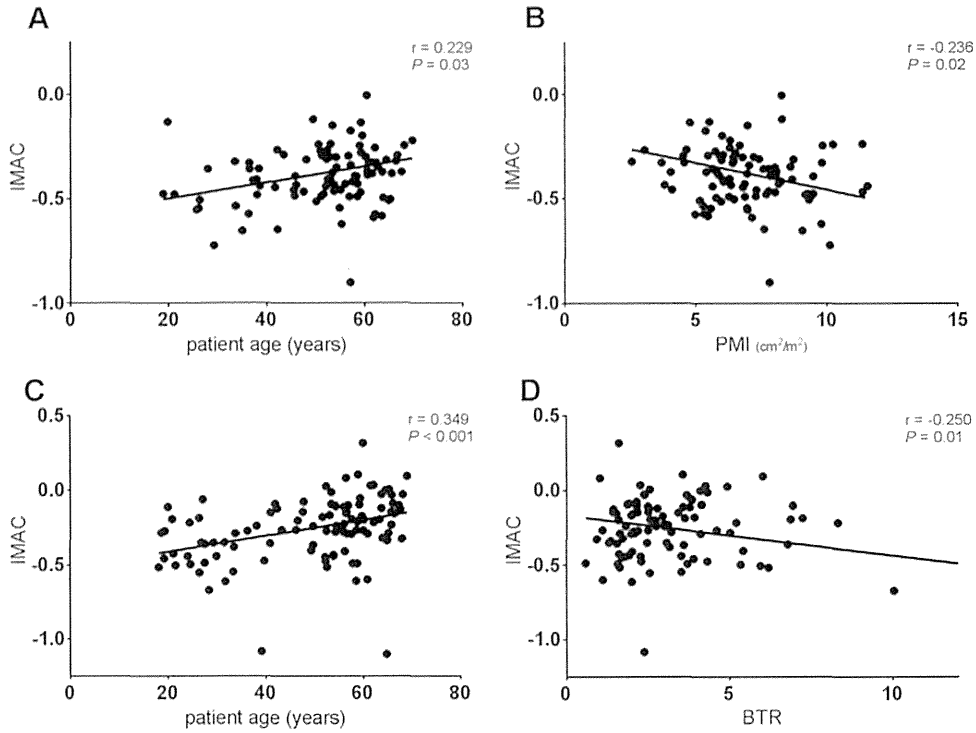

Figure 2. Correlations between IMAC and other factors. (A) For males, a significant positive relationship was observed between IMAC and patient age ($r = 0.229$, $P = 0.03$). (B) For males, a significant negative relationship was observed between IMAC and PMI ($r = -0.236$, $P = 0.02$). (C) For females, a significant positive relationship was observed between IMAC and age ($r = 0.349$, $P < 0.001$). (D) For females, a significant negative relationship was found between IMAC and BTR ($r = -0.250$, $P = 0.01$).

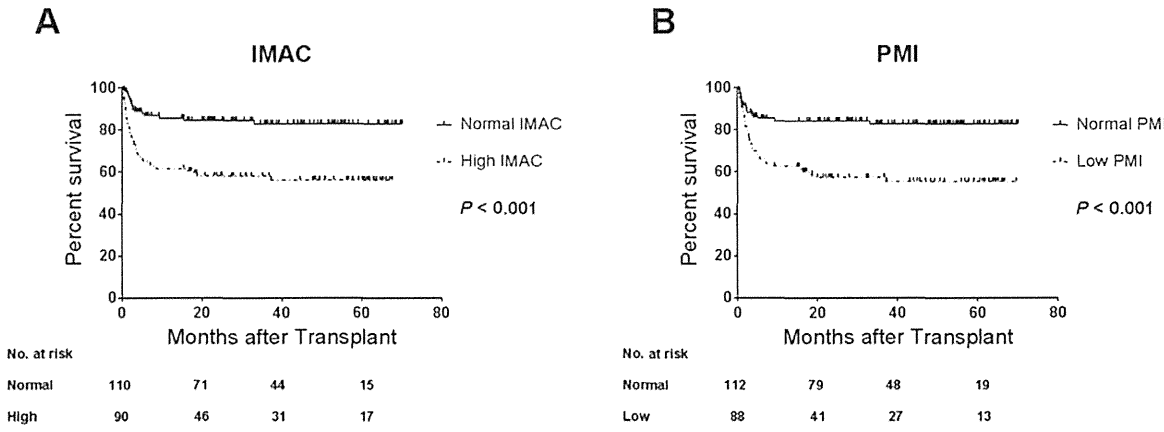

Figure 3. Overall survival rates according to IMAC and PMI. (A) The overall survival rate after LDLT was significantly lower in patients with high IMAC (n = 90) than in patients with normal/low IMAC (n = 110; $P < 0.001$). (B) The overall survival rate was significantly lower in patients with low PMI (n = 88) than in patients with normal/high PMI (n = 112; $P < 0.001$).

bleeding (n = 1); and others (n = 2). There were no significant differences in the causes of death between the 2 subgroups ($P = 0.78$). Similarly, between the 2 subgroups classified according to PMI, we found no significant differences in the causes of death ($P = 0.33$).

Risk Factors for Poor Survival in Patients Undergoing LDLT

Univariate analysis revealed that preoperative high IMAC and low PMI were significant risk factors for death after LDLT ($P < 0.001$ and $P < 0.001$, respectively) (Table 1). IMAC and PMI were so well correlated that multivariate analysis was performed incorporating either parameter. As a result, preoperative high IMAC and low PMI were identified as poor prognostic factors after LDLT (Table 2).

DISCUSSION

This retrospective study shows that preoperative IMAC and PMI were independent prognostic factors for overall survival in patients undergoing LDLT. In

TABLE 1. Univariate Analysis of Factors Affecting Posttransplantation Patient Survival

Variable	1-Year OS, n (%)	*P* Value
Recipient age (years)		
<50 (n = 71)	50 (70.4)	0.25
≥50 (n = 129)	101 (78.3)	
Donor age (years)		
<50 (n = 120)	95 (79.2)	0.11
≥50 (n = 80)	56 (70.0)	
Sex		
Male (n = 95)	72 (75.8)	0.72
Female (n = 105)	79 (75.2)	
Original disease		
HCC (n = 67)	53 (79.1)	0.27
HBV or HCV-associated LC (n = 38)	29 (76.3)	
PBC or PSC (n = 34)	20 (58.8)	
Others (n = 61)	49 (80.3)	
ABO compatibility		
Identical/compatible (n = 140)	108 (77.1)	0.23
Incompatible (n = 60)	43 (71.7)	
MELD score		
<20 (n = 125)	98 (78.4)	0.27
≥20 (n = 75)	53 (70.7)	
Child-Pugh classification		
A, B (n = 75)	60 (80.0)	0.39
C (n = 125)	91 (72.8)	
GRWR		
<0.8% (n = 60)	48 (80.0)	0.39
≥0.8% (n = 140)	103 (73.6)	
Graft		
Right (n = 107)	85 (107)	0.09
Left (n = 93)	66 (71.0)	
Operative time (hours)		
<12 (n = 50)	40 (80.0)	0.52
≥12 (n = 150)	111 (74.0)	
Operative blood loss (L)		
<10 (n = 140)	103 (73.6)	0.23
≥10 (n = 60)	48 (80.0)	
Pretransplant IMAC		
High (n = 90)	56 (62.2)	<0.001
Normal/low (n = 110)	95 (86.4)	
Pretransplant PMI		
Low (n = 88)	**56 (63.6)**	**<0.001**
Normal/high (n = 112)	**95 (84.8)**	

TABLE 2. Multivariate Analysis of Factors Affecting Posttransplantation Patient Survival

Variable	OR	95% CI	*P* Value
Left lobe graft	1.614	0.840-3.127	0.15
Pretransplant high IMAC	3.898	2.025-7.757	<0.001
Left lobe graft	1.532	0.797-2.960	0.20
Pretransplant low PMI	3.635	1.896-7.174	<0.001

patients with end-stage liver disease requiring LT, anthropometric parameters such as body mass index and arm muscle circumference are usually overestimated as a result of edema and ascites. In addition, biological markers such as prealbumin, albumin, and cholinesterase are not useful for evaluating patient nutritional status because these parameters are affected by underlying liver dysfunction. CT, magnetic resonance imaging, or dual energy X-ray absorptiometry (DXA) is often used to evaluate skeletal muscle mass. However, DXA measurement exposes patients to radiation, and DXA may overestimate muscle mass because muscle hydration or intramuscular fat deposition can be detected as lean tissue. Because BIA can easily and automatically measure whole-body skeletal muscle mass, BIA seems suitable for assessing the body composition and nutritional status of patients. However, in our previous study, 35.4% of the study patients (68/192) were excluded from analysis because they could not stand independently for more than 2 minutes because of their general condition or they could not undergo BIA for subemergent LT.[8] In the present study, to eliminate such selection bias for patient inclusion in the study group, we used preoperative CT images to evaluate sarcopenia, which allowed us to assess 85.1% of the patients (200/235) undergoing LDLT. The use of CT images in the present study allowed calculation of the volume and ROI of skeletal muscle, providing assessment of not only the quantity but also the quality of skeletal muscle. The European Working Group on Sarcopenia in Older People has recommended that the definition of sarcopenia include loss of not only skeletal muscle mass but also strength.[1] However, most previous studies have investigated only skeletal muscle mass to define sarcopenia because muscle strength and function have been difficult to evaluate. Recent evidence suggests that fat accumulation within skeletal muscle is associated with muscle weakness, poor function, and increased risk of incidental mobility limitations because it alters muscle fiber orientation and the force-producing capabilities of the whole muscle.[15-17] In addition, intramuscular adipose tissue secretes inflammatory cytokines such as tumor necrosis factor-α, leading to systemic inflammation that can inhibit muscle force production even in the absence of muscle atrophy.[18,19] Kitajima et al.[11] have investigated the relationship between IMAC and the severity of NASH. On the basis of these findings, we focused on intramuscular fat accumulation as a new parameter in evaluating preoperative sarcopenia, instead of measuring muscle strength, and we investigated the impact of IMAC on survival in patients undergoing LDLT.

We determined that there was a significant positive relationship between IMAC and patient age in both males and females, which supports previous findings that intramuscular adipose tissue increases with age.[9] We discovered that IMAC in males had a significant negative relationship with PMI ($r = -0.236$, $P = 0.02$), but IMAC in females did not ($r = 0.026$,

$P = 0.79$). We found a similar result in our analysis of healthy donors, which showed a significantly negative relationship between IMAC and PMI in male donors ($r = -0.342$, $P = 0.04$) but not in female donors ($r = -0.166$, $P = 0.38$). We speculated that this was because, in some patients with normal PMI, especially in females who had more adipose tissue than males, even though their amount of lean skeletal muscle mass is low, PMI could be calculated as normal because of a large amount of IMAT. Thus, measuring only PMI could not detect such patients as having sarcopenia, especially for females. For females, a significant negative relationship was observed between IMAC and BTR ($r = -0.250$, $P = 0.01$). For patients with LC, BCAA has been found to stimulate detoxification of ammonia to glutamine in skeletal muscle.[20-22] Therefore, we speculate that more BCAA is needed for detoxification of ammonia to glutamine in skeletal muscle with higher IMAC, which leads to a decrease in the BTR. However, these relationships are so different between the sexes that further investigations are needed on the composition of skeletal muscle, including the types of muscle fibers, the contribution of intramuscular adipose tissue to detoxification of ammonia, and the association between serum glutamine and ammonia level in LC.

The prognostic significance of sarcopenia has been reported for various kinds of diseases.[2-8] Montano-Loza et al.[23] also demonstrated that the presence of sarcopenia increased the risk of sepsis-related death in patients with cirrhosis, which might be due to impaired immunity. The present study showed no significant differences in the causes of death between patients with and without sarcopenia. However, the associations among immunity, inflammation, and adipocytokines such as adiponectin and leptin have recently been emphasized as the key mechanisms by which sarcopenia affects patient survival.[24,25] We speculate that, in sarcopenic patients, the inflammatory microenvironment and impaired immunity caused by the increase in adiposity could lead to an increased risk of mortality. We are now investigating the relationship between nutritional conditions, including IMAC, and immunological status, which affects the incidence of infections, sepsis, and post-transplant rejection.

We have previously performed LT even for the patients who were refused at other transplant centers for various reasons such as severe general conditions and ABO-incompatible donors because our institute is a tertiary liver transplant center in Japan. Most recently, we reported that pretransplant low skeletal muscle mass measured by BIA was an independent risk factor for death after LDLT.[8] We have added, on the basis of this finding, a new indication to our selection criteria for LT since January 2013: patients who can walk by themselves. Moreover, we are now conducting a prospective study to evaluate the relationship between preoperative IMAC and muscle strength or physical disability and the impact of preoperative IMAC on outcomes after LT. We consider that this investigation could allow development of new appropriate selection criteria for recipients of LDLT.

Several limitations must be borne in mind when considering the present study. First, the correlation of IMAC with skeletal muscle strength could not be investigated because this was a retrospective study. However, recent investigations have shown that intramuscular fat accumulation contributes to the decline of muscle strength and quality.[9,15-17] These findings support our idea that IMAC could be a new parameter for assessing sarcopenia instead of measuring solely muscle strength. In addition, we are now conducting a prospective study to evaluate preoperative sarcopenia that includes the measurement of grip strength; this investigation may reveal the relationship between IMAC and muscle strength. Second, we have to consider whether our cutoff values for IMAC and PMI were adequate to define sarcopenia. Until now, several reports have provided a definition of sarcopenia, but there is no criterion to define sarcopenia objectively.[26,27] In the present study, we determined the cutoff values of IMAC and PMI based on ROC curves. The use of ROC curves is a more accurate and objective method than the use of SD for the design of cutoff values. However, a significant positive relationship was observed between IMAC and patient age; therefore, it might be necessary to investigate the cutoff level in consideration of patient age. Further investigations will be necessary for this. Finally, we have to consider whether CT imaging at the umbilical level was adequate for the evaluation of skeletal muscle mass. In most previous studies, skeletal muscle mass and psoas muscle mass were evaluated from axial CT imaging at the third lumbar vertebrae (L3) level.[2-6] However, Durand et al.[28] recently measured transversal psoas muscle thickness (TPMT) on a CT image at the level of umbilicus and showed that TPMT/height might be predictive of mortality in patients with LC. The authors mentioned that, although the level of the umbilicus can be easily identified on CT scan, it might be difficult to identify a given lumbar section precisely because of sacralization of the L5 vertebrae, lumbar wedge fractures, and more pronounced lordosis in patients with refractory ascites. In addition, in original reports on IMAC by Kitajima et al.,[10,11] IMAC was calculated by CT imaging at the umbilical level. Moreover, we could find a significantly positive relationship between PMI and skeletal muscle mass measured by BIA in both males ($r = 0.635$, $P < 0.001$) and females ($r = 0.264$, $P = 0.04$). On the basis of these findings, PMI measured with preoperative CT imaging at the umbilical level could substitute for evaluation of whole-body muscle mass.

In conclusion, the quality and the quantity of skeletal muscle mass have been observed to be closely involved with posttransplantation mortality in patients undergoing LDLT. Preoperative quality and quantity of skeletal muscle could be incorporated into new selection criteria for LDLT. Perioperative nutritional therapy and rehabilitation could be important for good outcomes after LDLT.

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TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

LETTER TO THE EDITORS

Fluctuations in the concentration/dose ratio of calcineurin inhibitors after simeprevir administration in patients with recurrent hepatitis C after liver transplantation

doi:10.1111/tri.12438

Dear Sirs,

As the efficacy of dual therapy with peginterferon and ribavirin for recurrent hepatitis C after liver transplantation is limited, direct-acting antiviral agents (DAA) should be considered. First-generation NS3/4A inhibitors, such as telaprevir or boceprevir, for liver transplant recipients are problematic because of their inhibitory action on cytochrome P450 3A (CYP3A), an enzyme responsible for the metabolism of calcineurin inhibitors including tacrolimus and cyclosporine. In fact, administration of telaprevir resulted in elevation of blood concentrations and increase in the elimination half-life of calcineurin inhibitors (CNI) [1]. Therefore, when telaprevir is used for recurrent hepatitis C after liver transplantation, the dose of the CNI needs to be reduced to maintain proper blood concentrations, resulting in a significant increase in the concentration/dose (C/D) ratio of the CNI after the administration of telaprevir [2].

Since January 2014, we started using the second-generation NS3/4A inhibitor simeprevir along with peginterferon

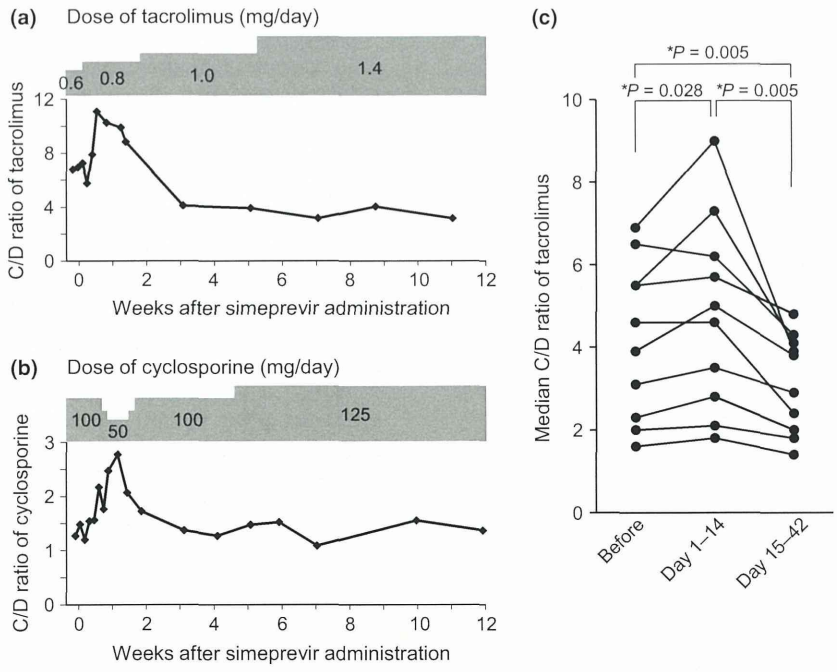

Figure 1 (a,b) Time course of the concentration/dose (C/D) ratio of a calcineurin inhibitor for case 1 (a) and case 2 (b), both of which involved triple therapy with simeprevir, peginterferon, and ribavirin for recurrent hepatitis C after liver transplantation. The fine line represents the C/D ratio of tacrolimus (ng/ml per mg) in a or cyclosporine (ng/ml per mg) in b. The dose of calcineurin inhibitors is shown in gray boxes. (c) Median C/D ratio of tacrolimus in 10 patients with simeprevir-based triple therapy after liver transplantation. Significant differences between the 2 groups are indicated by * with *P* values analyzed by Wilcoxon's signed-rank test. Difference among the 3 groups is also significant by Friedman's test ($P < 0.001$).

and ribavirin for patients with recurrent hepatitis C after liver transplantation. The dose of the CNI was adjusted using therapeutic drug monitoring (TDM) of either tacrolimus or cyclosporine. In 11 cases, we identified fluctuations in the C/D ratio during the simeprevir-based triple therapy. Six of the 11 patients were men, and the median age was 64 years (range, 46–73 years). Before the treatment, fibrosis scores F1 and F2 based on the METAVIR score was found in five and six patients, respectively. Tacrolimus-based immunosuppression with (n = 4) or without (n = 6) mycophenolate mofetil was administered to 10 patients, and cyclosporine with mycophenolate mofetil was administered to one patient. Median serum alanine aminotransferase (ALT) level before treatment was 51 IU/l (range, 21–115), and ALT of all patients decreased to the normal range in the first 2 weeks of treatment.

For the first 2 cases, the time course of the C/D ratio of tacrolimus in case 1 and cyclosporine in case 2 is shown in Fig. 1a and b. Blood concentrations of tacrolimus and cyclosporine were adjusted to trough levels 6–8 and 150–200 ng/ml, respectively, using TDM after simeprevir administration (100 mg/day). The C/D ratio of calcineurin inhibitors were elevated in the first 2 weeks in both cases, but decreased thereafter, necessitating an increase in the dose of the calcineurin inhibitor. The median C/D ratio of tacrolimus before, the first 2 weeks after, and 3–6 weeks after simeprevir administration in the 10 consecutive cases of patients receiving tacrolimus and simeprevir-based triple therapy in our hospital is shown in Fig. 1c. The median C/D ratio significantly increased from 4.25 ng/ml/mg before simeprevir administration to 4.8 ng/ml per mg in the first 2 weeks, but significantly decreased to 3.35 ng/ml per mg after 3 weeks of simeprevir administration.

These findings revealed the importance of TDM of CNI in transplant recipients undergoing simeprevir-based triple therapy. During the first 2 weeks, elevation of the C/D ratio would be caused by the interaction of simeprevir with CNI, because simeprevir is metabolized by the enzyme CYP3A, which is responsible for the metabolism of CNI. Notably, the C/D ratio was significantly decreased after 2 weeks of simeprevir-based triple therapy, despite continuous simeprevir administration at the same dose. The mechanism for the decrease in concentration of CNI with effective antiviral therapy has been proposed to be due to an increased metabolism of CNI by improvement in liver function [3]. Changes of CNI concentrations would be more dynamic using DAA, because of the drug–drug interaction and strong anti-HCV effect. Therefore, we should be cautious of the fluctuations in the CNI concentrations especially during DAA-based therapy and thus recommend TDM during the entire period of antiviral therapy.

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Funding

This work was supported by Labor Sciences Research Grants for Research on Hepatitis from the Ministry of Health, Labor and Welfare, Japan; and Astellas Pharma Inc.

Conflict of interest

Y Ueda and S Uemoto have received research grants from Astellas Pharma Inc.

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LIVER TRANSPLANTATION 20:1486–1496, 2014

ORIGINAL ARTICLE

Perioperative Changes in Nutritional Parameters and Impact of Graft Size in Patients Undergoing Adult Living Donor Liver Transplantation

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Derangements of various serum biochemical nutritional/metabolic parameters are common in patients with end-stage liver disease who undergo liver transplantation (LT). The aim of this study was to explain the benefit of LT with respect to parameter changes and to examine the impact of the graft-to-recipient weight ratio (GRWR) on such changes. We investigated each parameter's course in 208 adult recipients for 1 year after living donor LT and analyzed changes in the parameters with a GRWR of 0.8% as the cutoff point. Bonferroni corrections were applied to account for multiple testing. Liver disease–induced high pretransplant ammonia and tyrosine levels and low branched-chain amino acids to tyrosine ratio (BTR) and zinc levels normalized within 2 weeks after transplantation, and the total lymphocyte count (TLC) normalized within 2 months, whereas low pretransplant prealbumin levels took 1 year to normalize. Branched-chain amino acids (BCAA), zinc, and TLC levels transiently dropped shortly after transplantation and then were corrected later. An accelerated recovery of ammonia and tyrosine levels and the BTR were found with larger grafts, especially early after transplantation, whereas zinc, prealbumin, BCAA, and TLC levels recovered regardless of the graft size. In conclusion, graft size had little effect on the recovery of nutritional/metabolic parameters except for ammonia and tyrosine levels. *Liver Transpl 20:1486-1496, 2014.*

Received May 2, 2014; accepted August 25, 2014.

In patients with end-stage liver disease undergoing liver transplantation (LT), protein-energy malnutrition is common and negatively affects clinical outcomes in terms of posttransplant survival and complications.[1] Therefore, the instigation of specialized nutritional status measurements and interventions is required. Derangements of various serum biochemical nutritional parameters such as zinc, prealbumin, branched-chain amino acids (BCAA), tyrosine, and total lymphocyte count (TLC) and related metabolic parameters such as the BCAA to tyrosine ratio (BTR) and ammonia are not uncommon in these patients as a result of the debilitating hepatic pathology and its medical management.[2-6] These parameters could be good indicators of nutritional/metabolic status trends after LT. However, their posttransplant changes with respect to the preoperative levels remain unclear.

Abbreviations: ALF, acute liver failure; BCAA, branched-chain amino acids; BMI, body mass index; BTR, branched-chain amino acids to tyrosine ratio; CT, computed tomography; CTP, Child-Turcotte-Pugh; GRWR, graft-to-recipient weight ratio; HCC, hepatocellular carcinoma; LDLT, living donor liver transplantation; L/S, liver-to-spleen attenuation; LT, liver transplantation; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; POD, postoperative day; PSC, primary sclerosing cholangitis; TLC, total lymphocyte count; w1, week 1; w2, week 2; w3, week 3; w4, week 4.

Potential conflict of interest: Nothing to report.

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DOI 10.1002/lt.23992
View this article online at wileyonlinelibrary.com.
LIVER TRANSPLANTATION.DOI 10.1002/lt. *Published on behalf of the American Association for the Study of Liver Diseases*

Studies reporting amino acid levels after LT have been performed only in the early postoperative period[7] or without a definite time course.[8] It is, therefore, not clear whether levels of circulating amino acids are normalized in the clinically stable long-term course after LT. We hypothesize that successful LT would be sufficient to correct the disturbed amino acid metabolism found in liver cirrhosis.

In deceased donor LT, the graft size is sufficient for the recipient. In contrast, in living donor liver transplantation (LDLT), the graft size is small and is an important factor for posttransplant survival. However, there is relatively little information on the short- and long-term changes in nutritional/metabolic parameters after LDLT that could reflect the adequacy of the graft mass to provide sufficient metabolic and synthetic function, which is the key factor in the success of LDLT. Although partial liver grafts undergo a rapid regenerative response, with the largest changes in the liver volume occurring during the first week after transplantation, regeneration is suppressed in small-for-size grafts after LDLT. Thus, grafts with an inadequate graft-to-recipient weight ratio (GRWR) cannot meet the functional demand of the recipients.[9] On the other hand, some have concluded that smaller grafts are capable of regeneration to a greater extent and that the regenerative liver response is proportional to the amount of liver transplanted.[10,11] Yoshida et al.[7] presumed improvements in some nutritional parameters shortly after LDLT to be derived from the GRWR discrepancy. Our hypothesis is that the posttransplant recovery of nutritional/metabolic parameters, especially in the early period after grafting, might be affected by GRWR. To obtain insight into these questions, the present retrospective longitudinal study was performed to clarify the short- and long-term courses of circulating levels of the aforementioned parameters after successful adult LDLT and to analyze the impact of GRWR on such posttransplant changes in LDLT recipients.

PATIENTS AND METHODS

Patients

The study subjects were 208 adult patients (age $\geq$ 18 years) who underwent primary LDLT at Kyoto University Hospital between February 2008 and August 2012. There were 98 males and 110 females, and the median patient age was 54 years (range = 18-69 years). The patients provided written informed consent before the start of the study, which was approved by the ethics committee of Kyoto University in accordance with the Declaration of Helsinki of 1996.

The median Model for End-Stage Liver Disease (MELD) score was 19 (range = 6–55). Sixty-eight patients were ABO-incompatible, and 140 were identical or compatible. The Child-Turcotte-Pugh (CTP) classifications were C, B, and A for 139, 55, and 14 patients, respectively. The indications for LT were hepatocellular carcinoma (HCC; n = 52), hepatocellular diseases such as hepatitis B or C virus–associated liver cirrhosis (n = 46), progressive intrahepatic cholestatic diseases including primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC; n = 34), acute liver failure (ALF; n = 15), biliary atresia after the Kasai procedure (n = 14), alcoholic liver cirrhosis (n = 11), metabolic liver diseases (n = 7), nonalcoholic steatohepatitis (NASH; n = 7), autoimmune hepatitis (n = 4), and other causes (n = 18).

Donor and Graft Selection

The selection criteria for donors and grafts have been described elsewhere.[12,13] Briefly, according to computed tomography (CT) scan volumetric analysis, the liver weight was calculated, and the graft type was selected. If necessary, portosystemic collateral ligation with splenectomy was performed for the prevention of the steal phenomenon after LT as well as the control of the portal venous pressure ($\leq$15 mm Hg). This allowed the lower limit of GRWR to be safely reduced to 0.6% and a left lobe graft to be used whenever feasible.[13,14] All grafts used had a liver-to-spleen attenuation (L/S) ratio on CT $\geq$ 1.1 to exclude grafts with hepatic steatosis > 30%.[15]

Surgical Procedures and Immunosuppressive Treatments

The selection criteria for the recipients as well as surgical and back-table techniques for the donors and recipients have been described in detail elsewhere.[16-18] Orthotopic adult LDLT was performed with a right lobe graft for 102 patients, with a left lobe graft for 100 patients, with a posterior segment graft for 5 patients, and with a whole liver graft as a domino graft from a patient with familial amyloid polyneuropathy for 1 patient. Each graft was perfused with cold histidine tryptophan ketoglutarate (0°C-4°C; Custodiol, Essential Pharmaceuticals, LLC, Newtown, PA). Immediately after the perfusion of the preservation solution, all resected liver grafts were measured. The actual graft weight was used for the calculation of GRWR. The median GRWR was 0.89% (range = 0.53%-1.50%). At the time of surgery in all recipients, a tube jejunostomy for enteral nutrition was placed in the proximal jejunum with a 9-Fr enteral tube.

The baseline immunosuppressive regimen consisted of tacrolimus or cyclosporine and low-dose steroids, as described elsewhere.[19] Patients who were ABO-incompatible also underwent preoperative plasma exchange to reduce A/B antibodies to 1:8 or more and received 300 mg of intravenous rituximab (anti-CD20 monoclonal antibody) approximately 2 weeks before LT. A hepatic artery infusion of prostaglandin E1 and methylprednisolone was started at the time of the surgery and was continued for 21 and 7 days, respectively, and this was followed by oral mycophenolate mofetil (500 mg twice daily).[20] All patients received intravenous antimicrobial prophylaxis with

ampicillin (0.5 g) and cefotaxime (0.5 g) twice daily for 3 days; this started 30 minutes before surgery.

Perioperative Nutritional Therapy

Preoperative nutritional therapy was administered for approximately 2 weeks before LDLT and consisted of the following components: a nutrient mixture enriched with BCAA (Aminoleban EN, Otsuka Pharmaceutical Co., Tokyo, Japan) or BCAA nutrients (Livact, Ajinomoto Pharmaceuticals Co., Ltd., Tokyo, Japan) as a late-evening snack, synbiotics with a supplementation product (GFO, Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) 3 times daily, a lactic fermented beverage once per day, and polaprezinc (Promac D, Zeria Pharmaceutical Co., Ltd., Tokyo, Japan) for patients with low zinc, as described previously.[21] Dietitians adjusted the type and amount of food for each patient to maintain a total daily caloric intake of 35 to 40 kcal/kg and a protein intake of 1.2 to 1.5 g/kg, including BCAA nutrients, according to the guidelines of the European Society of Parenteral and Enteral Nutrition.[22] We could not perform preoperative nutritional therapy for patients with ALF due to emergent LT, and also BCAA supplementation was unsuitable for those patients already having elevated plasma amino acids.[23]

Formulas containing BCAA and polaprezinc were discontinued after LDLT, and early postoperative enteral nutrition was started within the first 24 hours after surgery through the tube jejunostomy with an immune-modulating enteral diet enriched with hydrolyzed whey peptide (MEIN, Meiji Dairies Co., Tokyo, Japan); its composition[21] and its administration protocol[24,25] have been previously described. Oral nutrition was started after the ability to swallow was regained, usually on approximately postoperative day (POD) 5. Dietitians calculated the daily amounts of protein and carbohydrates required for each recipient and the speed of the enteral nutrition accordingly. Enteral feeding was stopped when adequate oral intake containing solid food was tolerated. For synbiotics, all patients received the aforementioned supplementation product 3 times daily and a lactic fermented beverage once per day via the feeding tube or orally until discharge.

Analyzed Parameters

Preoperative and postoperative laboratory parameters were retrospectively reviewed from the clinical charts of the recipients. The standard reference intervals for these parameters at our institute were as follows: zinc, 65 to 110 μg/dL; prealbumin, 20 to 40 mg/dL; TLC, 1200 to 3200/μL; ammonia, 20 to 60 μg/dL; BCAA, 344 to 713 μmol/L; tyrosine, 53 to 98 μmol/L; and BTR, 4.41 to 9.3. A longitudinal study was performed to examine peritransplant changes in the aforementioned parameters at the following serial time points: before the operation at admission (baseline); PODs 2, 3, and 5; week 1 (w1), week 2 (w2), week 3 (w3), and week 4 (w4); months 2, 3, and 6; and year 1 after transplantation. The baseline pretransplant level of each parameter was statistically compared with its counterparts at each time point in the posttransplant observation (follow-up) period, and the degree of significance of each comparison was plotted on graphs.

A statistical comparison was performed for the level of each parameter at each of the assigned peritransplant time points between recipients of grafts with a GRWR $< 0.8\%$ (n = 67) and recipients of grafts with a GRWR $\geq 0.8\%$ (n = 141). There were 31 patients who received grafts with a GRWR $< 0.7\%$ and 36 patients who received grafts with a GRWR between 0.7% and 0.8%. We also compared peritransplant parameters among recipients who were stratified as follows: those with a GRWR $< 0.7\%$ (n = 31), those with a GRWR between 0.7% and 0.8% (n = 36), and those with a GRWR $\geq 0.8\%$ (n = 141).

Perioperative changes in parameters were compared among patients with preoperative CTP classification A (n = 14), B (n = 55), or C (n = 139) at various time points and also among patients with diseases of other etiologies who received preoperative nutritional therapy (n = 193) and those with ALF (n = 15) who did not. We also compared nutritional recovery between ABO-incompatible recipients (n = 68) and ABO-compatible recipients (n = 140). Moreover, we examined these preoperative nutritional/metabolic parameters as risk factors for posttransplant mortality.

Statistical Analysis

Data were summarized as means and standard deviations for continuous variables. Continuous variables (or parameters) were nonparametrically analyzed with the Wilcoxon signed-rank test to assess postoperative changes from the preoperative state, whereas other comparisons, including those of groups with GRWR $<$ 0.8% and GRWR $\geq$ 0.8% at each time point, were compared with the Mann-Whitney U test or the one-way analysis of variance as appropriate. Two-tailed P values were corrected for multiple testing with the Bonferroni method[26] (with the statistical significance set at $P < 0.0045$, where $0.0045 = 0.05/11$) for 11 tests within each independent family of comparisons of each single parameter performed at the designated 11 peritransplant time points. Only the adjusted P values are presented here. Categorical variables were compared with the χ^2 test or Fisher's exact test as appropriate. The survival rate was calculated via Kaplan-Meier methods, with differences evaluated by log-rank testing. Any variable identified as significant ($P < 0.05$) in the univariate analysis was considered a candidate for the multivariate analysis using multiple logistic regression models. All statistical data were generated in JMP 5.0.1 (SAS Institute, Cary, NC) and Prism 6.02 (GraphPad Software, Inc., La Jolla, CA).

RESULTS

The 1-, 2-, 3-, 4-, and 5-year cumulative survival rates of the whole cohort were 72.6%, 69.2%, 68.1%,