right-lobe living donor liver transplantation. Hepatobiliary Pancreat Dis Int 2010;9:135-138.

11. Umeda Y, Yagi T, Sadamori H, Matsukawa H, Matsuda H, Shinoura S, et al. Effects of prophylactic splenic artery modulation on portal overperfusion and liver regeneration in small-for-size graft. Transplantation 2008;86:673-680.
12. Yoshizumi T, Taketomi A, Uchiyama H, Harada N, Kayashima H, Yamashita Y, et al. Graft size, donor age, and patient status are the indicators of early graft function after living donor liver transplantation. Liver Transpl 2008;14:1007-1013.
13. Hori T, Ogura Y, Ogawa K, Kaido T, Segawa H, Okajima H, et al. How transplant surgeons can overcome the inevitable insufficiency of allograft size during adult living-donor liver transplantation: strategy for donor safety with a smaller-size graft and excellent recipient results. Clin Transplant 2012;26:E324-E334.
14. Kaido T, Mori A, Ogura Y, Hata K, Yoshizawa A, Iida T, et al. Lower limit of the graft-to-recipient weight ratio can be safely reduced to 0.6% in adult-to-adult living donor liver transplantation in combination with portal pressure control. Transplant Proc 2011;43:2391-2393.
15. Iwasaki M, Takada Y, Hayashi M, Minamiguchi S, Haga H, Maetani Y, et al. Noninvasive evaluation of graft steatosis in living donor liver transplantation. Transplantation 2004;78:1501-1505.
16. Morioka D, Egawa H, Kasahara M, Ito T, Haga H, Takada Y, et al. Outcomes of adult-to-adult living donor liver transplantation: a single institution's experience with 335 consecutive cases. Ann Surg 2007;245:315-325.
17. Inomata Y, Uemoto S, Asonuma K, Egawa H. Right lobe graft in living donor liver transplantation. Transplantation 2000;69:258-264.
18. Takada Y, Ueda M, Ito T, Sakamoto S, Haga H, Maetani Y, et al. Living donor liver transplantation as a second-line therapeutic strategy for patients with hepatocellular carcinoma. Liver Transpl 2006;12:912-919.
19. Inomata Y, Tanaka K, Egawa H, Uemoto S, Ozaki N, Okajima H, et al. The evolution of immunosuppression with FK506 in pediatric living-related liver transplantation. Transplantation 1996;61:247-252.
20. Egawa H, Ohdan H, Haga H, Tsuruyama T, Oike F, Uemoto S, Ozawa K. Current status of liver transplantation across ABO blood-type barrier. J Hepatobiliary Pancreat Surg 2008;15:131-138.
21. Kaido T, Ogawa K, Fujimoto Y, Ogura Y, Hata K, Ito T, et al. Impact of sarcopenia on survival in patients undergoing living donor liver transplantation. Am J Transplant 2013;13:1549-1556.
22. Plauth M, Cabré E, Riggio O, Assis-Camilo M, Pirlich M, Kondrup J, et al.; for ESPEN (European Society for Parenteral and Enteral Nutrition). ESPEN guidelines on enteral nutrition: liver disease. Clin Nutr 2006;25:285-294.
23. Shimomura Y, Murakami T, Nagasaki M, Honda T, Goto H, Kotake K, et al. Regulation of branched-chain amino acid metabolism and pharmacological effects of branched-chain amino acids. Hepatol Res 2004;30S:3-8.
24. Kaido T, Mori A, Ogura Y, Hata K, Yoshizawa A, Iida T, et al. Impact of enteral nutrition using a new immunomodulating diet after liver transplantation. Hepatogastroenterology 2010;57:1522-1525.
25. Kaido T, Mori A, Oike F, Mizumoto M, Ogura Y, Hata K, et al. Impact of pretransplant nutritional status in patients undergoing liver transplantation. Hepatogastroenterology 2010;57:1489-1492.
26. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310:170.
27. Pescovitz MD, Mehta PL, Jindal RM, Milgrom ML, Leapman SB, Filo RS. Zinc deficiency and its repletion following liver transplantation in humans. Clin Transplant 1996;10:256-260.
28. Raut V, Uemoto S. Management of ABO-incompatible living-donor liver transplantation: past and present trends. Surg Today 2011;41:317-322.
29. Montano-Loza AJ. Clinical relevance of sarcopenia in patients with cirrhosis. World J Gastroenterol 2014;20:8061-8071.
30. Holecek M. Branched-chain amino acids and ammonia metabolism in liver disease: therapeutic implications. Nutrition 2013;29:1186-1191.
31. Shanbhogue RL, Bistrian BR, Lakshman K, Crosby L, Swenson S, Wagner D, et al. Whole body leucine, phenylalanine, and tyrosine kinetics in end-stage liver disease before and after hepatic transplantation. Metabolism 1987;36:1047-1053.
32. Urano E, Yamanaka-Okumura H, Teramoto A, Sugihara K, Morine Y, Imura S, et al. Pre- and postoperative nutritional assessment and health-related quality of life in recipients of living donor liver transplantation. Hepatol Res; doi:10.1111/hepr.12263.

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,

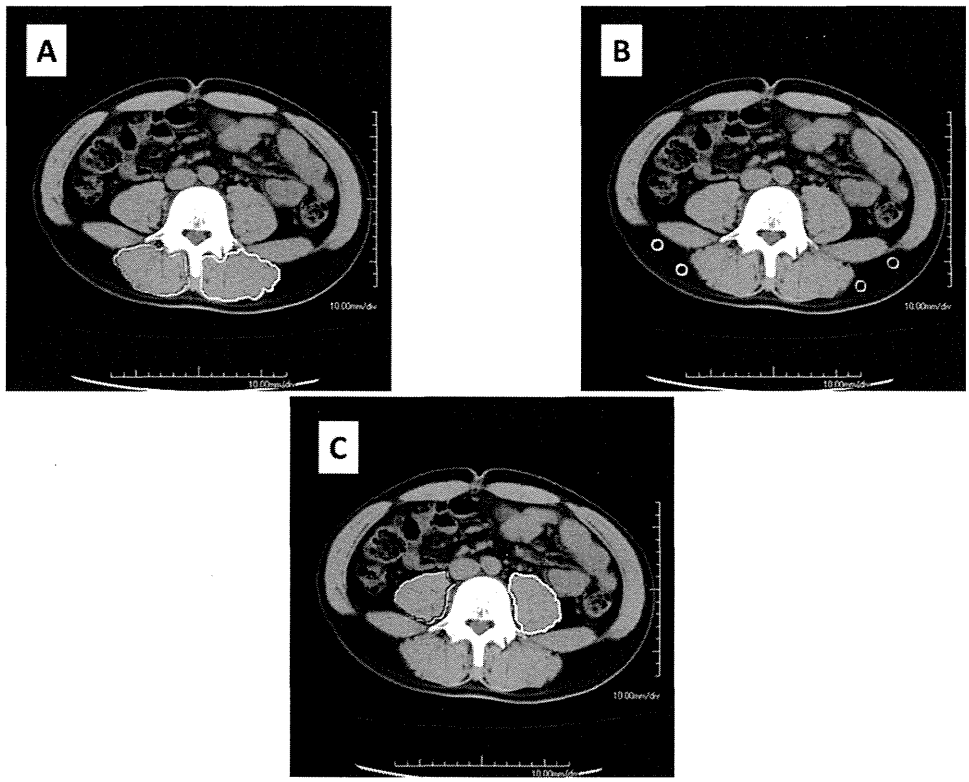

Figure 1. Cross-sectional CT images at the umbilical level. (A) Subfascial muscular tissue in the multifidus muscle was precisely traced. (B) Four small circles were placed on subcutaneous fat away from major vessels. ROIs of the multifidus muscle and the subcutaneous fat (Hounsfield units) were measured 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

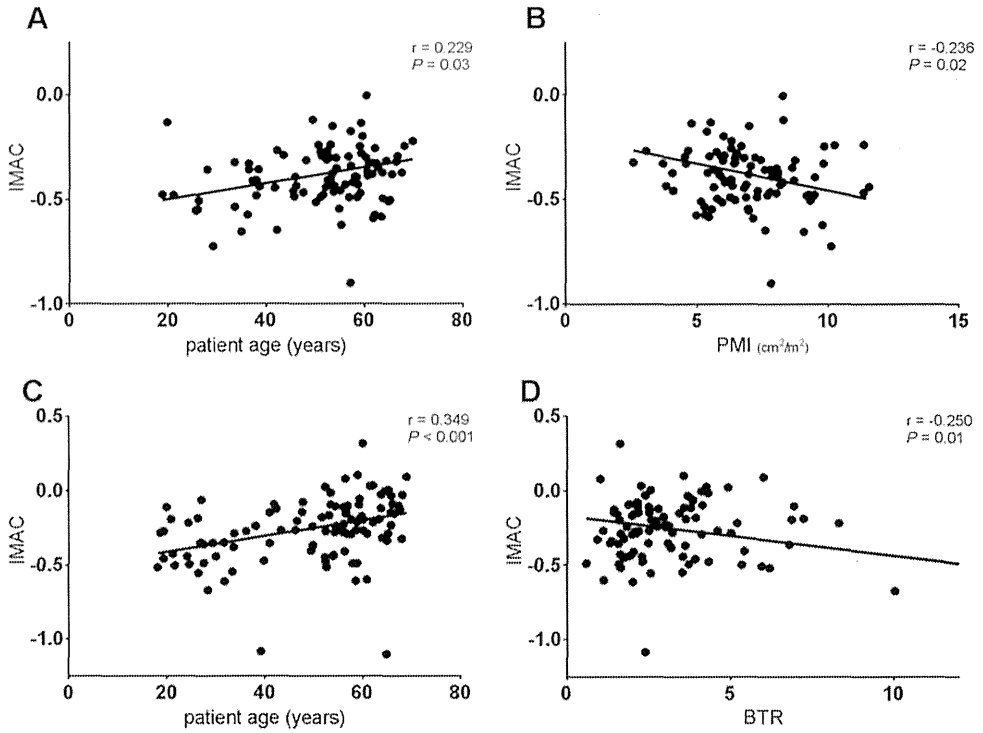

Figure 2. Correlations between IMAC and other factors. (A) For males, a significant positive relationship was observed between IMAC and patient age ($r = 0.229$, $P = 0.03$). (B) For males, a significant negative relationship was observed between IMAC and PMI ($r = -0.236$, $P = 0.02$). (C) For females,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

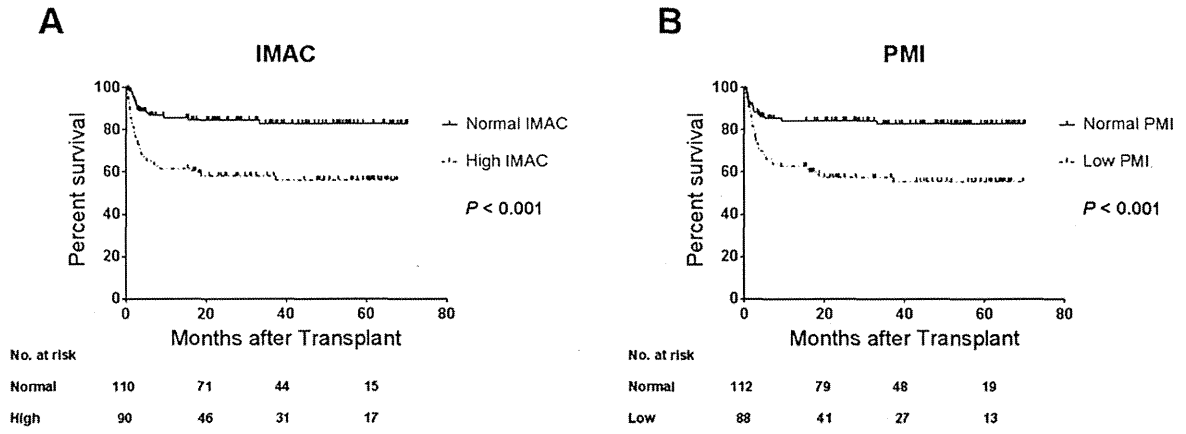

Figure 3. Overall survival rates according to IMAC and PMI. (A) The overall survival rate after LDLT was significantly lower in patients with high IMAC (n = 90) than in patients with normal/low IMAC (n = 110; $P < 0.001$). (B) The overall survival rate was significantly lower in patients with low PMI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

REFERENCES

1. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al.; for European Working Group on Sarcopenia in Older People. Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. Age Ageing 2010;39:412-423.
2. Peng PD, van Vledder MG, Tsai S, de Jong MC, Makary M, Ng J, et al. Sarcopenia negatively impacts short-term outcomes in patients undergoing hepatic resection for colorectal liver metastasis. HBP (Oxford) 2011;13:439-446.
3. van Vledder MG, Levolger S, Ayez N, Verhoef C, Tran TC, Ijzermans JN. Body composition and outcome in patients undergoing resection of colorectal liver metastases. Br J Surg 2012;99:550-557.
4. Peng P, Hyder O, Firoozmand A, Kneuertz P, Schulick RD, Huang D, et al. Impact of sarcopenia on outcomes following resection of pancreatic adenocarcinoma. J Gastrointest Surg 2012;16:1478-1486.
5. Meza-Junco J, Montano-Loza AJ, Baracos VE, Prado CM, Bain VG, Beaumont C, et al. Sarcopenia as a prognostic index of nutritional status in concurrent cirrhosis and hepatocellular carcinoma. J Clin Gastroenterol 2013;47: 861-870.
6. Harimoto N, Shirabe K, Yamashita YI, Ikegami T, Yoshizumi T, Soejima Y, et al. Sarcopenia as a predictor of prognosis in patients following hepatectomy for hepatocellular carcinoma. Br J Surg 2013;100:1523-1530.
7. Englesbe MJ, Patel SP, He K, Lynch RJ, Schaubel DE, Harbaugh C, et al. Sarcopenia and mortality after liver transplantation. J Am Coll Surg 2010;211:271-278.
8. Kaido T, Ogawa K, Fujimoto Y, Ogura Y, Hata K, Ito T, et al. Impact of sarcopenia on survival in patients undergoing living donor liver transplantation. Am J Transplant 2013;13:1549-1556.
9. Marcus RL, Addison O, Kidde JP, Dibble LE, Lastayo PC. Skeletal muscle fat infiltration: impact of age, inactivity, and exercise. J Nutr Health Aging 2010;14:362-366.
10. Kitajima Y, Eguchi Y, Ishibashi E, Nakashita S, Aoki S, Toda S, et al. Age-related fat deposition in multifidus muscle could be a marker for nonalcoholic fatty liver disease. J Gastroenterol 2010;45:218-224.
11. Kitajima Y, Hyogo H, Sumida Y, Eguchi Y, Ono N, Kuwashiro T, et al.; for Japan Nonalcoholic Fatty Liver Disease Study Group (JSG-NAFLD). Severity of nonalcoholic steatohepatitis is associated with substitution of adipose tissue in skeletal muscle. J Gastroenterol Hepatol 2013;28:1507-1514.
12. Inomata Y, Uemoto S, Asonuma K, Egawa H. Right lobe graft in living donor liver transplantation. Transplantation 2000;69:258-264.
13. Ito T, Kiuchi T, Egawa H, Kaihara S, Oike F, Ogura Y, et al. Surgery-related morbidity in living donors of right-lobe liver graft: lessons from the first 200 cases. Transplantation 2003;76:158-163.
14. Morioka D, Egawa H, Kasahara M, Ito T, Haga H, Takada Y, et al. Outcomes of adult-to-adult living donor liver transplantation: a single institution's experience with 335 consecutive cases. Ann Surg 2007;245:315-325.
15. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. J Appl Physiol 2000;89:104-110.
16. Visser M, Kritchevsky SB, Goodpaster BH, Newman AB, Nevitt M, Stamm E, Harris TB. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. J Am Geriatr Soc 2002;50:897-904.
17. Delmonico MJ, Harris TB, Visser M, Park SW, Conroy MB, Velasquez-Mieyer P, et al.; for Health, Aging, and Body. Longitudinal study of muscle strength, quality, and adipose tissue infiltration. Am J Clin Nutr 2009;90:1579-1585.
18. Hardin BJ, Campbell KS, Smith JD, Arbogast S, Smith J, Moylan JS, Reid MB. TNF-alpha acts via TNFR1 and muscle-derived oxidants to depress myofibrillar force in murine skeletal muscle. J Appl Physiol 2008;104:694-699.
19. Schaap LA, Pluijm SM, Deeg DJ, Harris TB, Kritchevsky SB, Newman AB, et al. Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength. J Gerontol A Biol Sci Med Sci 2009;64:1183-1189.
20. Dam G, Keiding S, Munk OL, Ott P, Buhl M, Vilstrup H, et al. Branched-chain amino acids increase arterial blood ammonia despite enhanced intrinsic muscle ammonia metabolism in patients with cirrhosis and healthy subjects. Am J Physiol Gastrointest Liver Physiol 2011;301: G269-G277.
21. Dam G, Ott P, Aagaard NK, Vilstrup H. Branched-chain amino acids and muscle ammonia detoxification in cirrhosis. Metab Brain Dis 2013;28:217-220.
22. Holecek M. Branched-chain amino acids and ammonia metabolism in liver disease: therapeutic implications. Nutrition 2013;29:1186-1191.
23. Montano-Loza AJ, Meza-Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, Sawyer MB. Muscle wasting is associated with mortality in patients with cirrhosis. Clin Gastroenterol Hepatol 2012;10:166-173.
24. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 2006;6:772-783.
25. Lutz CT, Quinn LS. Sarcopenia, obesity, and natural killer cell immune senescence in aging: altered cytokine levels as a common mechanism. Aging 2012;4:535-546.
26. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. J Am Geriatr Soc 2002;50:889-896.
27. Chien MY, Huang TY, Wu YT. Prevalence of sarcopenia estimated using a bioelectrical impedance analysis prediction equation in community-dwelling elderly people in Taiwan. J Am Geriatr Soc 2008;56:1710-1715.
28. Durand F, Buyse S, Francoz C, Laouénan C, Bruno O, Belghiti J, et al. Prognostic value of muscle atrophy in cirrhosis using psoas muscle thickness on computed tomography. J Hepatol 2014;60:1151-1157.

Surg Today (2014) 44:2293–2299
DOI 10.1007/s00595-014-0839-y

ORIGINAL ARTICLE

Impact of preoperative uncontrollable hepatic hydrothorax and massive ascites in adult liver transplantation

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Received: 23 April 2013 / Accepted: 16 December 2013 / Published online: 8 February 2014

Abstract
Purpose Uncontrollable hepatic hydrothorax and massive ascites (H&MA) requiring preoperative drainage are sometimes encountered in liver transplantation (LT). We retrospectively analyzed the characteristics of such patients and the impact of H&MA on the postoperative course.
Methods We evaluated 237 adult patients who underwent LT in our institute between April 2006 and October 2010.
Results Recipients with uncontrollable H&MA (group HA: $n = 36$) had more intraoperative bleeding, higher Child–Pugh scores, lower serum albumin concentrations and higher blood urea nitrogen concentrations than those without uncontrollable H&MA (group C: $n = 201$). They were also more likely to have preoperative hepatorenal syndrome and infections. The incidence of postoperative bacteremia was higher (55.6 vs. 46.7 %, $P = 0.008$) and the 1- and 3-year survival rates were lower (1 year: 58.9 vs. 82.9 %; 3 years: 58.9 vs. 77.7 %; $P = 0.003$) in group HA than in group C. The multivariate proportional regression analyses revealed that uncontrollable H&MA and the Child–Pugh score were independent risk factors for the postoperative prognosis.
Conclusions Postoperative infection control may be an important means of improving the outcome for patients with uncontrollable H&MA undergoing LT, and clinicians should strive to perform surgery before H&MA becomes uncontrollable.

Keywords Hepatic hydrothorax · Liver transplantation · Massive ascites · Bacteremia · Mortality

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Introduction

Liver transplantation (LT) is now performed in many countries as a treatment for end-stage liver disease. As a result of expansion of the indications for LT for HCC candidates, it also provides a potentially curative treatment for unresectable hepatocellular carcinoma, and satisfactory long-term outcomes have been achieved [1]. However, LT still has a relatively high mortality rate compared with other hepatobiliary-pancreatic procedures owing to the potentially poor preoperative condition of the patients, the use of immunosuppressive agents, and the development of rejection and infection. The preoperative condition of transplant recipients is a particularly important factor that influences the outcome of LT.

Patients requiring LT sometimes present with uncontrollable hepatic hydrothorax and massive ascites (H&MA) that must be drained before surgery; it has also been reported that H&MA is an independent risk factor for postoperative bacteremia [2].

In this study, we focused on preoperative uncontrollable H&MA in LT candidates, and evaluated the perioperative course of patients who went on to become LT recipients.

Methods

Patients

Between April 2006 and October 2010, 237 adult patients underwent LT at Kyoto University Hospital, Japan (227 were living-donor cases and 10 were deceased-donor cases). There were 117 males and 120 females; their median age was 54.9 years (range 18–69 years). The indications for LT in these patients included hepatocellular carcinoma in 78 cases; hepatocellular diseases, such as hepatitis B virus-associated liver cirrhosis, hepatitis C virus-associated liver cirrhosis and alcoholic liver cirrhosis in 133 cases; progressive intrahepatic cholestatic diseases, such as primary biliary cirrhosis and primary sclerosing cholangitis in 29 cases; re-transplantation owing to graft loss in 17 cases; fulminant hepatic failure in 17 cases; cryptogenic cirrhosis in 14 cases; biliary atresia after the Kasai operation in eight cases; autoimmune hepatitis in six cases; metabolic liver diseases in six cases and other causes in seven cases.

Antimicrobial prophylaxis

The perioperative antimicrobial prophylaxis consisted of cefotaxime (2 g/day intravenously) and ampicillin (4 g/day intravenously) twice daily for 72 h starting 30 min before surgery. Laxatives were administered as bowel preparation.

The therapeutic antibiotics were usually determined based on the results of culturing samples taken from infection sites or blood. When the focus of the infection was unknown, broad-spectrum antibiotics were administered empirically. Pre-transplant, antibiotics were given to treat ongoing infections, such as spontaneous bacterial peritonitis or pneumonia.

Immunosuppression

The standard immunosuppression protocol comprised tacrolimus and a low-dose steroid. We endeavored to maintain the whole blood trough level of tacrolimus between 10 and 15 ng/mL during the first 2 weeks, around 10 ng/mL during the next 2 weeks and between 5 and 8 ng/mL thereafter. For the recipients who suffered from side effects due to the tacrolimus treatment, we changed the immunosuppressant from tacrolimus to cyclosporine microemulsion.

Steroid therapy with methylprednisolone sodium succinate was initiated at a dose of 10 mg/kg before graft reperfusion and then tapered from 1 mg/kg/day on day 1 to 0.3 mg/kg/day by the end of the first month; this was followed by 0.1 mg/kg/day until the end of the third month. Steroid administration was terminated thereafter. In the event of postoperative infection, steroids were discontinued, and the target trough level of tacrolimus was decreased.

Study design

The medical records of patients undergoing LT were examined retrospectively to identify the recipients who had required preoperative drainage due to uncontrollable H&MA (allocated to group HA; $n = 36$) and those who had not (allocated to a control group [group C]; $n = 201$). The recipients' demographic details, surgical data, occurrence of preoperative hepatorenal syndrome (HRS) and postoperative bacteremia, and patient survival were recorded and compared between the groups. Finally, the independent prognostic factors for the patient survival were evaluated by the multivariate analyses.

Indications for thoracic or intraperitoneal drainage

We considered refractory uncontrollable massive ascites as that meeting the criteria for grade 3 with diuretic resistance, as defined by the International Ascites Club [3]. The diagnosis of hepatic hydrothorax was based on evidence of a large volume effusion (estimated to be >500 ml) on chest radiography and/or computed tomography (CT) scans in the absence of underlying pulmonary or cardiac diseases [4].

We used the following indications to guide decisions about when to drain H&MA before surgery: (1) patients with hydrothorax who remained hypoxic with a peripheral oxygen saturation (SpO_2) of ≤95 % despite supplemental oxygen administration underwent thoracic drainage to improve their respiratory function; (2) patients with hepatorenal syndrome (HRS) underwent peritoneal or thoracic drainage with intravenous albumin supplementation to normalize their hemodynamic parameters and prevent the progression of renal dysfunction and (3) patients experiencing dyspnea, difficulty eating and drinking or abdominal pain underwent thoracic or peritoneal drainage for symptomatic relief.

Hepatorenal syndrome was diagnosed according to the criteria of the International Ascites Club [5] as follows: (1) a low glomerular filtration rate, indicated by serum creatinine >1.5 mg/dl or 24-h creatinine clearance <40 ml/min; (2) the absence of shock, ongoing bacterial infections and recent or current treatment with nephrotoxic drugs; (3) no sustained improvement of renal function by diuretic withdrawal and intravenous administration of fluids and (4) the absence of significant proteinuria (<500 mg/day) and ultrasonographic abnormalities in the kidneys.

Infections and bacteremia were defined using the criteria proposed by the Centers for Disease Control and Prevention and based on our previous report regarding LT patients [2]. The diagnosis of infection in ascitic fluid or pleural effusion, including spontaneous bacterial peritonitis (SBP) and spontaneous bacterial empyema (SBEM), was based on the level of polymorphonuclear white cells (>250/mm^3 with positive culture or >500/mm^3 if culture was negative) [6, 7].

Thoracic and intraperitoneal drainage were not indicated as a treatment for SBEM and SBP in general, and antimicrobial therapy was started immediately instead of drainage. In cases where SBEM or SBP had been diagnosed after the initiation of drainage, drainage was maintained in combination with antimicrobial therapy.

The study protocol was approved by the Medical Ethics Committee of Kyoto University, and the study was performed in accordance with the ethical standards established in the 1975 Declaration of Helsinki.

Drainage protocol

We used fine catheters (Argyle™ aspiration Seldinger kit, 5Fr; COVIDIEN Japan, Shizuoka, Japan) for thoracic and intraperitoneal drainage to avoid injuring collateral vessels. Before performing drainage, Doppler ultrasonography and CT were used to establish the location of any abnormal intercostal or abdominal wall collateral vessels so as to avoid hemorrhage. To prevent hypotension, depletion of protein and electrolytes, and re-expansion pulmonary edema, the rapid drainage of ascites and pleural effusion was avoided. Initially, 1,000 ml was drained, and then the drainage volume was gradually increased from the second day. At the same time, intravenous fluid and albumin replacement was undertaken during the drainage.

Statistical analysis

The values are presented as the means and standard deviations (SD) unless otherwise indicated. Continuous data were analyzed by Student's t test or the Mann–Whitney test, while categorical data were analyzed with the Chi-square test. For the survival analyses, Kaplan–Meier survival curves were constructed and analyzed by the log-rank test, and the multivariate analyses of survival were performed by the proportional regression hazard analyses. Variables identified as significant ($P < 0.05$) in the univariate analyses were considered to be candidates for the multivariate analyses. Values of $P < 0.05$ were considered to be significant. The statistical analyses were performed using the Prism version 5 software program (GraphPad Software Inc., San Diego, USA) for the univariate analyses and the JMP version 9 software program (SAS institute Inc., Cary, NC, USA) for the multivariate analyses.

Results

Details of preoperative drainage management

Preoperative thoracic or intraperitoneal drainage was performed in 36 patients (15.2 %), all of whom later underwent scheduled living-donor LT. In 16 cases, thoracic drainage was required (including five cases with SBEM), intraperitoneal drainage was performed in 15 cases (including eight cases of SBP); and both thoracic drainage and intraperitoneal drainage were needed in five cases (including two cases of SBP). The median drainage period was 13 days (range 1–33 days) for thoracic drainage and 9 days (1–44 days) for intraperitoneal drainage.

Infectious complications related to the placement of an intraperitoneal drainage tube occurred in three patients (8.3 %), while there were no complications related to the placement of a thoracic drainage tube. Infections were diagnosed 2, 6 and 7 days after the placement of the drainage catheter; patients were treated with antibiotics and a new catheter was re-sited. There were no other complications, such as hemothorax or pneumothorax.

Patient characteristics

Table 1 shows the characteristics of the groups; there were no significant differences in the sex, recipient age, blood type compatibility, graft-recipient weight ratio (GRWR), length of the operation, cold and warm ischemic times, model for end-stage liver disease (MELD) score, evidence of preoperative hepatic encephalopathy or preoperative serum creatinine of the patients in each group. Group HA was characterized by a higher intraoperative blood loss ($P = 0.02$), higher Child–Pugh score ($P = 0.001$), lower preoperative serum albumin concentration ($P = 0.01$) and higher serum blood urea nitrogen concentration ($P = 0.003$) compared with group C.

Preoperative HRS and perioperative infections

Group HA had a significantly higher incidence of HRS than group C (nine out of 36 cases [25 %] vs. 20 out of 201 cases [9.9 %], $P = 0.017$), and a significantly higher incidence of preoperative infections (19 out of 36 cases [52.8 %] vs. 35 out of 201 cases [17.4 %], $P = 0.0001$). The incidence of bacteremia within 90 days of LT was significantly higher in group HA than group C (20 out of 36 cases [55.6 %] vs. 94 out of 201 cases [46.7 %], $P = 0.008$).

Table 1 Background and characteristics of the two groups

	Group HA (n = 36)	Group C (n = 201)	P
Sex (male/female)	18/18	99/102	0.54
Age	55.3 ± 7.89	51.1 ± 12.81	0.05
ABO compatibility	Identical/compatible 28 Incompatible 8	Identical/compatible 153 Incompatible 48	0.51
Graft type	Right 15, left 19 Posterior 2	Right 114, left 71 Posterior 6, whole 10	0.08
GRWR	0.90 ± 0.17	0.98 ± 0.31	0.14
Operation time (min)	814.8 ± 120.5	793.6 ± 149.4	0.45
Blood loss (ml)	12,244.2 ± 9,505.48	8,814.4 ± 7,251.1	0.02
CIT (min)	98.1 ± 53.3	119.1 ± 113.0	0.31
WIT (min)	43.6 ± 13.2	48.6 ± 53.4	0.60
MELD score	20.9 ± 8.84	20.5 ± 9.47	0.79
Child–Pugh score	11.4 ± 2.20	10.0 ± 2.22	0.001
Preop hepatic encephalopathy	1.44 ± 0.65	1.32 ± 0.65	0.1517
Preop serum Alb (g/dl)	2.65 ± 0.42	2.94 ± 0.52	0.001
Preop serum BUN (mg/dl)	28.9 ± 19.4	18.9 ± 14.3	0.0003
Preop serum Cr (mg/dl)	1.17 ± 0.68	1.19 ± 3.58	0.98

GRWR graft-recipient weight rate, *CIT* cold ischemic time, *WIT* warm ischemic time, *MELD* model for end-stage liver disease, *Preop* preoperative, *Alb* albumin, *BUN* blood urea nitrogen, *Cr* creatinine

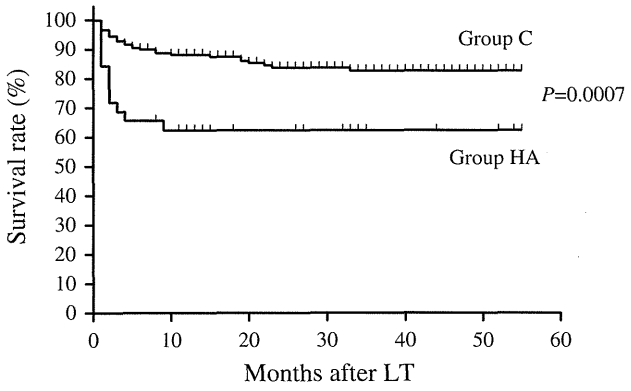

Fig. 1 The survival rates of patients with (group HA) and without (group C) uncontrollable H&MA. The cumulative survival rates after LT were significantly lower in group HA than in group C (P = 0.0007)

Postoperative mortality

Figure 1 shows the Kaplan–Meier survival curves of each group. The cumulative survival rates at 1 and 3 years after LT were both significantly lower in group HA than group C (1-year survival: 58.9 vs. 82.9 %; 3-year survival: 58.9 vs. 77.7 %, respectively; P = 0.003). The survival was worse in the HA group regardless of whether the patient had undergone intrathoracic or intraperitoneal drainage (Fig. 2a, b). Even when cases of infectious H&MA were excluded, those with sterile preoperative H&MA (n = 23) had a significantly worse prognosis than those in group C (1-year survival: 64.6 vs. 88.1 %; 3-year survival: 64.1 vs. 82.6 %, respectively; P = 0.015; Fig. 3).

When we subdivided the patients in group HA into two groups based on where there was a diagnosis of postoperative bacteremia, we found that the 1- and 3-year survival rates were significantly lower in those who developed bacteremia compared with those who did not (1-year survival: 41.2 vs. 86.2 %; 3-year survival: 41.2 vs. 86.2 %, respectively; P = 0.008).

Prognostic indicators after LT

The intraoperative blood loss, Child–Pugh score, preoperative albumin and blood urea nitrogen concentrations, HRS, preoperative infection and GRWR were included in the multivariate analysis, along with preoperative uncontrollable H&MA. We found that preoperative uncontrollable H&MA (hazard ratio: 2.304; P = 0.034) and the Child–Pugh score (hazard ratio: 1.258; P = 0.003) were independent risk factors for mortality after LT (Table 2).

Discussion

We analyzed the incidence and characteristics of patients with uncontrollable H&MA before LT and evaluated its effect on the postoperative course after LT.

Hepatic hydrothorax is thought to occur secondary to the passage of ascites through a diaphragmatic defect. Therefore, we included patients with hepatic hydrothorax and those with massive ascites in the same group. When we subdivided these patients into two groups based on the type of drainage, the survival rates were almost the same.

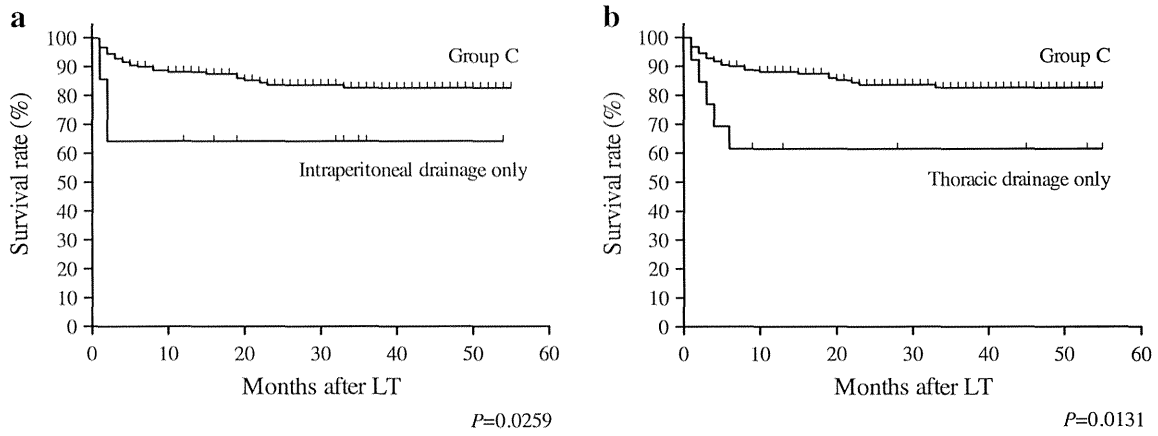

Fig. 2 The survival rates of patients depending on the site of drainage. Patients who underwent intraperitoneal drainage (**a**) and those who underwent thoracic drainage (**b**) had higher mortality rates than those in group C

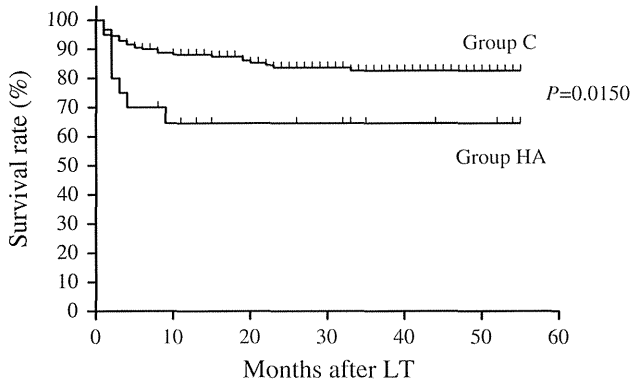

Fig. 3 Kaplan–Meier curves comparing group HA and group C after excluding the infectious H&MA cases from group HA. The mortality rates were still significantly higher in group HA than in group C

Table 2 Independent prognostic risk factors for mortality after liver transplantation

Variable	Hazard ratio	95 % Confidence interval	*P*
Preop uncontrollable hydrothorax and massive ascites	2.304	1.069–4.691	0.0339
Blood loss	1.000	0.999–1.000	0.1123
Child–Pugh score	1.258	1.085–1.422	0.0033
Preop serum Alb	1.003	0.460–2.144	0.9938
Preop serum BUN	1.004	0.983–1.024	0.7006
HRS	1.345	0.434–3.633	0.5884
Preop infection	1.088	0.493–2.270	0.8281
GRWR	0.971	0.269–3.036	0.9612

Preop preoperative, *H&MA* hydrothorax and massive ascites, *Alb* albumin, *BUN* blood urea nitrogen, *GRWR* graft-recipient weight ratio

Ascites is attributed to impaired albumin production in the liver, portal hypertension and salt retention owing to renal dysfunction. These symptoms are usually treated with a high-protein diet and diuretics, but in some cases, the ascites proves to be refractory to manipulating the dose and type of diuretics and dietary intake. A vicious cycle may develop in which increasing abdominal distension further impairs the hepatic function. Ascites and hydrothorax can cause SBP and SBEM, respectively, and can also cause a decrease in the circulating blood volume, which can lead to HRS. A transhepatic intra-jugular porto-systemic shunt (TIPS) is one of the options for treating refractory hepatic hydrothorax and massive ascites, and there are reports that TIPS is superior to large volume paracentesis in the control of ascites or hydrothorax [8, 9]. However, this treatment only provides supportive care and cannot prolong survival.

It is well recognized that the pre-transplant health of an LT recipient is closely associated with the postoperative mortality. Our study showed that patients with preoperative uncontrollable H&MA had a higher mortality rate after LT. The causes of death were mainly related to postoperative infections, including bacteremia. Notably, when cases of infectious H&MA were excluded, the remaining recipients with uncontrollable H&MA still had a poorer survival than those in group C. This finding suggests that LT recipients with uncontrollable H&MA are at risk of post-transplant mortality, regardless of the presence of preoperative infection. It is likely that the recipients in group HA were more severely compromised by more severe end-stage liver disease. The higher rate of postoperative infections in group HA might also be a consequence of the poorer general condition and comorbidities of the patients with high Child–Pugh scores. The substantially reduced survival rates in the patients in group HA diagnosed with postoperative infections suggests that effective postoperative

infection control could be a crucial means of improving the outcome after LT.

The MELD scores were not substantially different between the groups in our study. The MELD score is a useful means of prioritizing the waiting list, but it is controversial as to whether it can effectively predict the survival after LT [10–12]. The multivariate proportional hazard analyses in the present study revealed that uncontrollable H&MA was an independent risk factor for postoperative mortality. Somsouk et al. [13] reported that patients with moderate ascites and a MELD score <21 were at higher risk of death while on the waiting list for LT. It is possible that the presence of preoperative uncontrollable H&MA may be a more important prognostic indicator than the MELD scores. Clinicians should therefore carefully consider the timing of LT, undertaking transplantation before H&MA becomes uncontrollable.

Xiol et al. [14] and Serste et al. [15] have reported that the presence of preoperative hepatic hydrothorax had no significant negative influence on the postoperative outcome after deceased-donor LT. Xiol et al. [14] reported that the survival rate of patients with hydrothorax was 70 % at 8 years. However, in their hydrothorax group, they included not only patients with refractory hydrothorax, but also those with previous episodes of spontaneous bacterial empyema and those with uncomplicated hydrothorax with impaired hepatic function. In addition, the Child–Pugh score in their hydrothorax group was 9.9 ± 1.4, which was lower than that in our study (mean: 11.5). Serste et al. [15] established two control groups: a group with ascites but not hydrothorax, and a group with no ascites or hydrothorax, and compared the survival among the three groups. They found no significant differences in the overall risk of death, but the 1-year survival rate in the hydrothorax group was 64 ± 15 %, which was higher than expected. The apparent discrepancy in these findings regarding the impact of hydrothorax may also be a consequence of the type of LT. Most of the cases in the studies by Xiol and Serste [14, 15] were deceased-donor LT cases, while all of our cases received grafts from living donors. As the graft volume is limited in living-donor LT, the H&MA may have persisted due to higher portal venous pressures and hypoalbuminemia resulting from the inadequate postoperative hepatic synthetic function.

It has still not been established whether thoracic and/or intraperitoneal drainage is the best means of managing uncontrollable H&MA for liver cirrhosis (LC) before scheduled LT. However, complications related to paracentesis have been reported in only about 1 % of patients with coagulopathy [16]. Therefore, intraperitoneal drainage appears to be a safe approach. According to the treatment guidelines for LC [17, 18], intraperitoneal drainage is an effective first-line treatment for uncontrollable tense and refractory ascites. Total paracentesis reduces the intra-abdominal, intrathoracic, right arterial and pulmonary pressures, improving cardiac output by increasing the stroke volume without changing the heart rate [19]. Moreover, it results in a rapid decrease in portal pressure by decreasing the wedged hepatic venous pressure, and hence the hepatic venous pressure gradient [20]. Although intraperitoneal drainage is an established treatment for uncontrollable massive ascites, there are no data on its role in the management of SBP [18].

Regarding the management of refractory hydrothorax, thoracic drainage using a chest tube should be avoided due to the risk of complications [21]. It has been reported that chest tube insertion for hepatic hydrothorax carries significant morbidity and mortality, with questionable benefit [22, 23]. However, in our institution, the morbidity was 8.3 %, and all morbidities were related to intraperitoneal drainage. We experienced no serious or fatal complications, such as hemothorax or pneumothorax.

Drainage of H&MA might adversely influence a patient's preoperative condition. For example, the drainage of fluid could cause electrolyte and hemodynamic disturbance, and impair renal function. This can be prevented by adequate volume replacement with an appropriate combination of intravenous fluids. Nevertheless, the antibodies and immunocompetent cells in the hydrothorax and ascitic fluid cannot be replaced, which might result in a state of relative immunodeficiency, thus increasing the rate of postoperative infections.

Thoracic and intraperitoneal drainages alone without LT will not improve the prognosis of patients with end-stage liver diseases, but we should aim to improve the recipient's condition as much as possible before LT, especially if living-donor procedures are to be performed. We have recently ensured that the drainage of ascites or hydrothorax is not undertaken during the 3 days before LT in an effort to avoid intraperitoneal infections.

There are several limitations associated with our study. These are that it was a retrospective, single-center study, and the numbers of patients are small. We believe that a larger series and a multicenter study design would address these issues.

In conclusion, uncontrollable H&MA was found to be an independent risk factor for a poor post-transplant outcome in our study. In particular, for patients with uncontrollable H&MA, effective postoperative treatment of infections is a key to improving the outcome after LT. In addition, the timing of transplant is crucial; efforts should be made to perform surgery before H&MA becomes uncontrollable.

Conflict of interest The authors have no conflicts of interest to declare in association with this study.

References

1. Taketomi A, Shirabe K, Toshima T, Hashimoto N, Kayashima H, Ikegami T, et al. The long-term outcomes of patients with hepatocellular carcinoma after living donor liver transplantation: a comparison of right and left lobe grafts. Surg Today. 2012;42:559–64.
2. Iida T, Kaido T, Yagi S, Yoshizawa A, Hata K, Mizumoto M, et al. Post-transplant bacteremia in adult living donor liver transplant recipients. Liver Transpl. 2010;16:1379–85.
3. Arroyo V, Gines P, Gerbes AL, Dudley FJ, Gentilini P, Laffi G, et al. Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International Ascites Club. Hepatology. 1996;23:164–76.
4. Morrow CS, Kantor M, Armen RN. Hepatic hydrothorax. Ann Intern Med. 1958;49:193–203.
5. Gines P, Arroyo V. Hepatorenal syndrome. J Am Soc Nephrol. 1999;10:1833–9.
6. Rimola A, Garcia-Tsao G, Navasa M, Piddock LJ, Planas R, Bernard B, et al. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. International Ascites Club. J Hepatol. 2000;32:142–53.
7. Xiol X, Castellvi JM, Guardiola J, Sese E, Castellote J, Perello A, et al. Spontaneous bacterial empyema in cirrhotic patients: a prospective study. Hepatology. 1996;23:719–23.
8. Salerno F, Merli M, Riggio O, Cazzaniga M, Valeriano V, Pozzi M, et al. Randomized controlled study of TIPS versus paracentesis plus albumin in cirrhosis with severe ascites. Hepatology. 2004;40:629–35.
9. Sanyal AJ, Genning C, Reddy KR, Wong F, Kowdley KV, Benner K, et al. The North American Study for the treatment of refractory ascites. Gastroenterology. 2003;124:634–41.
10. Hayashi PH, Forman L, Steinberg T, Bak T, Wachs M, Kugelmas M, et al. Model for end-stage liver disease score does not predict patient or graft survival in living donor liver transplant recipients. Liver Transpl. 2003;9:737–40.
11. Ishigami M, Honda T, Okumura A, Ishikawa T, Kobayashi M, Katano Y, et al. Use of the model for end-stage liver disease (MELD) score to predict 1-year survival of Japanese patients with cirrhosis and to determine who will benefit from living donor liver transplantation. J Gastroenterl. 2008;43:363–8.
12. Selzner M, Kashfi A, Cattral MS, Selzner N, McGilvray ID, Greig PD, et al. Live donor liver transplantation in high MELD score recipients. Ann Surg. 2010;251:153–7.
13. Somsouk M, Kornfield R, Vittinghoff E, Inadomi JM, Biggins SW. Moderate ascites identifies patients with low model for end-stage liver disease scores awaiting liver transplantation who have a high mortality risk. Liver Transpl. 2011;17:129–36.
14. Xiol X, Tremosa G, Castellote J, Gornals J, Lama C, Lopez C, et al. Liver transplantation in patients with hepatic hydrothorax. Transpl Int. 2005;18:672–5.
15. Serste T, Moreno C, Francoz C, Razek WA, Paugham C, Belghitti J, et al. The impact of preoperative hepatic hydrothorax on the outcome of adult liver transplantation. Eur J Gastroenterol Hepatol. 2010;22:207–12.
16. Runyon BA. Paracentesis of ascitic fluid. A safe procedure. Arch Intern Med. 1986;146:2259–61.
17. Runyon BA, Committee APG. Management of adult patients with ascites due to cirrhosis: an update. Hepatology. 2009;49:2087–107.
18. Moore KP, Aithal GP. Guidelines on the management of ascites in cirrhosis. Gut. 2006;55(Suppl 6):vi1–12.
19. Pozzi M, Osculati G, Boari G, Serboli P, Colombo P, Lambrughi C, et al. Time course of circulatory and humoral effects of rapid total paracentesis in cirrhotic patients with tense, refractory ascites. Gastroenterology. 1994;106:709–19.
20. Luca A, Feu F, Garcia-Pagan JC, Jimenez W, Arroyo V, Bosch J, et al. Favorable effects of total paracentesis on splanchnic hemodynamics in cirrhotic patients with tense ascites. Hepatology. 1994;20:30–3.
21. Cardenas A, Arroyo V. Management of ascites and hepatic hydrothorax. Best Pract Res Clin Gastroenterol. 2007;21:55–75.
22. Orman ES, Lok AS. Outcomes of patients with chest tube insertion for hepatic hydrothorax. Hepatol Int. 2009;3:582–6.
23. Liu LU, Haddadin HA, Bodian CA, Sigal SH, Korman JD, Bodenheimer HC Jr, et al. Outcome analysis of cirrhotic patients undergoing chest tube placement. Chest. 2004;126:142–8.

Pretransplant replacement of donor liver grafts with recipient Kupffer cells attenuates liver graft rejection in rats

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jgh.12872

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Running title: Attenuated rejection by KC replacement

ABSTRACT

Background and Aim: Rejection of liver grafts is a difficult issue that has not been resolved. Preoperative replacement of liver cells in the graft with cells from the intended recipient may attenuate rejection. We investigated whether preoperative transplantation of recipient BMCs to the donor replaced liver allograft cells and attenuated rejection.

Methods: We used a rat model of allogeneic liver transplantation (LT) from DA to LEW rats. In BMC group, DA rats received BMC transplants from LacZ-transgenic Lewis rats at 1 week before LT. In Control group, DA rats received no preoperative treatment. We evaluated graft damage at 7 d after LT and the survival of the recipient rats.

Results: Rats in the BMC group experienced prolonged survival that was abrogated by the administration of gadolinium chloride to donors at 24 h before LT. Serum concentrations of total bilirubin and hyaluronic acid on day 7 were significantly lower in the BMC group, and histopathological analyses revealed that rejection of the liver graft was attenuated. X-gal staining and Immunohistostaining of the liver graft revealed that BMCs engrafted in the sinusoidal space differentiated into Kupffer cells.

Conclusions: Preoperative transplantation of recipient BMCs to liver transplant donors replaced donor KCs and attenuated post-LT rejection, indicating that this strategy may increase the success of liver transplantation.

Keywords: Chimerism, Bone Marrow Cells, Kupffer cells, Liver Transplantation, Xenotransplantation.

Introduction

Improvements in immunosuppressants, surgical techniques, and donor-recipient selection have increased the success rate of liver transplantation (LT). The gap between the numbers of patients requiring a liver transplant and the numbers of available organs is therefore increasing [1]. Thus, the shortage of donors remains a critical problem despite expansion of the donor pool and the efficient use of grafts in procedures such as split LT [2, 3]. Because of this, the death rates remain high for patients with end-stage liver disease awaiting a liver graft [4].

Xenotransplantation is expected to overcome the shortage of donors. Xenotransplantation of a baboon heart to a human neonate was attempted in 1983 [5], and two baboon-to-human [6,7] and one pig-to-human LTs [8] were performed in the United States in 1992–1993. However, to our knowledge, there have been no further attempts to perform clinical organ xenotransplantation. One important disadvantage of liver xenotransplantation is the intractable rejection of cells expressing xenogeneic antigens. Although the use of disparate species is hindered by intractable immunological barriers and involves ethical, societal, and