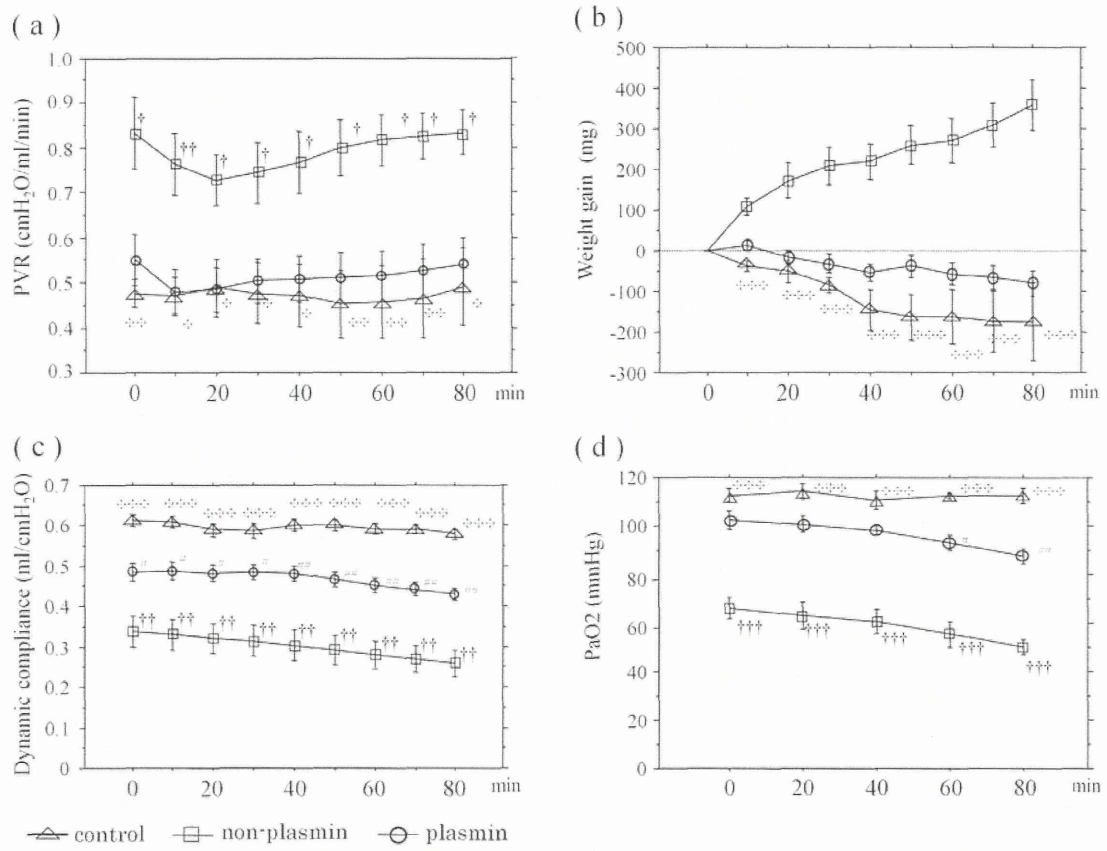

Figure 4 Physiologic lung function during reperfusion. (a) Pulmonary vascular resistance, (b) weight gain, (c) dynamic compliance and (d) PaO_2. $^{*}p < 0.05$, $^{**}p < 0.01$ or $^{***}p < 0.001$, between the control (triangles) and non-plasmin (squares) groups. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ or $^{\dagger\dagger\dagger}p < 0.001$ between the non-plasmin and plasmin groups (circles). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ or $^{\#\#\#}p < 0.001$ between the control and the plasmin groups. Data are expressed as mean ± SEM (BL, baseline).

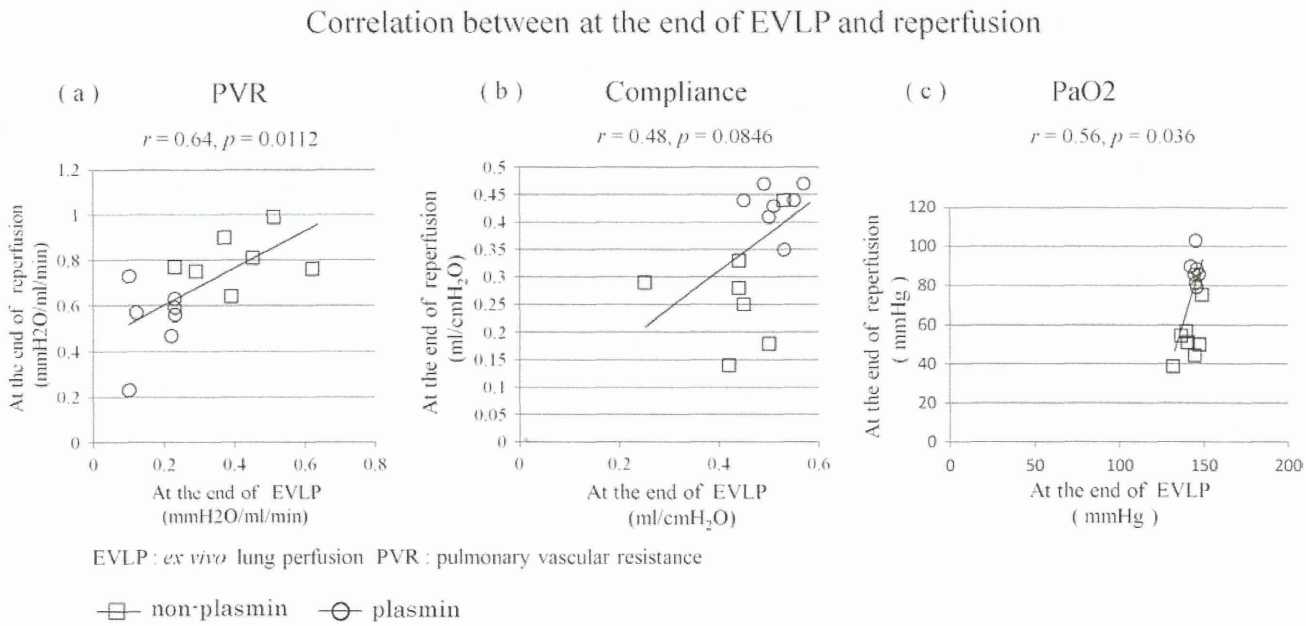

Figure 5 Correlation between physiologic lung function at the end of EVLP and lung functions 80 minutes after transplantation. EVLP, ex vivo lung perfusion. (a) Lung oxygenation at the end of EVLP was correlated with lung oxygenation 80 minutes after transplantation ($r = 0.64$, $p = 0.0112$). (b) There was no significant correlation between the dynamic pulmonary compliance at the end of EVLP and that 80 minutes after reperfusion ($r = 0.48$, $p = 0.0846$). (c) Although the range of PaO_2 during EVLP was very narrow (131.5 to 148.9 mm Hg), there was a slight correlation between the PaO_2 at the end of ELP and that 80 minutes after reperfusion ($r = 0.56$, $p = 0.036$).

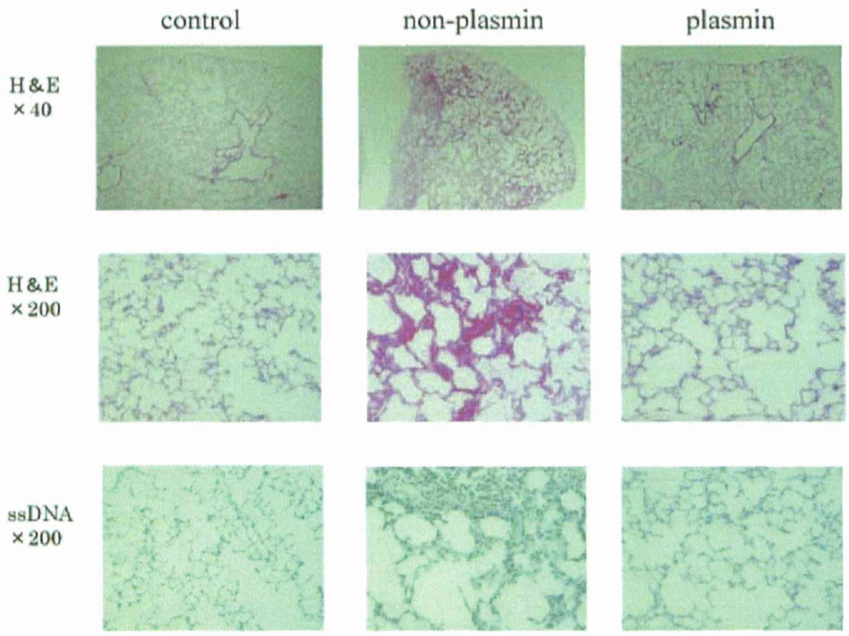

Figure 6 Histologic findings after EVLP (H&E staining and single-strand DNA staining).

We have previously reported on the efficacy of plasmin administration to the EVLP perfusate.[8] However, the physiologic data after reperfusion are still unclear. It is known that ischemia induces macrophages and endothelial cells to produce reactive oxygen species, cytokines, nicotinamide adenine dinucleotide phosphate oxidase and cell adhesion molecules.[11,12] Activation of neutrophils and upregulation of cell adhesion molecules on endothelial cells induces neutrophils to transmigrate into inflammatory sites.[22,23] In addition, platelet activation is induced after reperfusion. These sequential changes increase PVR and pulmonary capillary permeability, resulting in lung edema after reperfusion.[11,12]

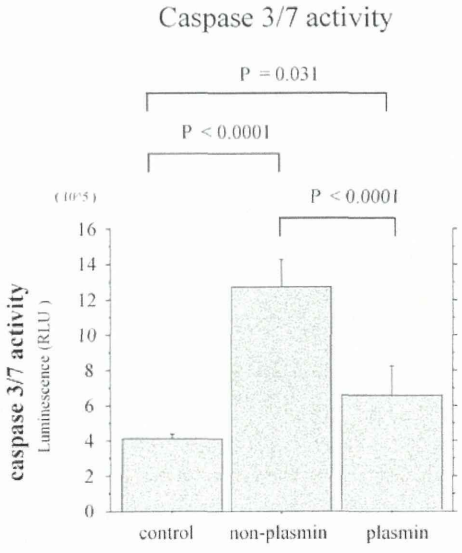

Figure 7 Caspase-3 and -7 activity assay. The caspase-3/-7 activity of the plasmin group was lower than that of the non-plasmin group (control: 4.1×10^5; non-plasmin: 12.8×10^5; non-plasmin: 6.5×10^5; control vs non-plasmin: $p < 0.0001$; non-plasmin vs plasmin: $p < 0.0001$; plasmin vs control: $p = 0.030$).

The perfusate in EVLP does not contain white blood cells, platelets and red blood cells; therefore, evaluation in EVLP could differ from that after reperfusion. To simulate the clinical settings of lung transplantation, the lung was stored at 4°C for 90 minutes after warm ischemia and reperfused with rat blood for 80 minutes.

Unlike our previous study, plasmin was administered during low perfusion flow because we believe that plasmin is more effectively administered in low flow rather than than high flow. Furthermore, to prevent vascular damage, it may be important to add plasmin as early as possible. In this study, during EVLP, the difference in physiologic data between the plasmin and non-plasmin groups was not very large; however, almost all data after reperfusion were significantly different between these two groups. The histologic findings in the non-plasmin group showed severe edema and alveolar hemorrhage. We detected many apoptotic cells with ssDNA staining and the results of caspase activity assay corresponded with these findings. Interestingly, the peripheral area where we found large amounts of residual thrombus after EVLP in our previous study was severely damaged after reperfusion. Initially, we believed that thrombus in the lung reduced the vascular bed and increased vascular pressure, causing lung edema in the normal area. Our findings from this study, however, strongly suggest that the peripheral lung area, with a pulmonary artery obstructed by thrombus, was latently damaged by insufficient perfusion during EVLP in the non-plasmin group. Even so, the area normally perfused was not severely damaged, showing a minor difference in the physiologic data between groups. After reperfusion, thrombus in the donor lung was resolved by plasminogen in the blood and the malperfusion areas were reperfused. Then, after reperfusion, severe damage was strongly induced by inflow of white blood cells and platelets into this area, which resulted in significant data differences between the groups.

In this study we observed a correlation between the physiologic data at the end of EVLP and that at the end of reperfusion. This significant finding may open the possibility that we can predict the behavior of the lung after reperfusion by physiologic data during EVLP. In fact, PVR at the end of EVLP showed a significant correlation with that at the end of reperfusion, which means that PVR could be a useful parameter for detecting damage by the thrombus and for evaluating the effects of fibrinolytic treatment. Although there was also a slight correlation between PaO_2 at the end of EVLP and that at 80 minutes after EVLP, the range of PaO_2 was very narrow. Because acellular solution contains no erythrocytes, the solution is saturated immediately, even in the edematous condition. Therefore, acellular EVLP cannot detect the difference in PaO_2 between the two groups. Yeung et al also demonstrated that PaO_2 cannot detect lung damage with acellular solution.[24] We also believe that PaO_2 is not a useful parameter for prediction of lung function after reperfusion. Dynamic compliance is considered to be among the most sensitive parameters for evaluation of the lungs in EVLP.[24] Although dynamic compliance at the end of EVLP tended to correlate with that at 80 minutes after reperfusion, it was not significant in this study. However, compliance after reperfusion was worse than that expected by compliance during EVLP. As described earlier, after reperfusion, malperfused areas were damaged by white blood cells after reperfusion, resulting in severe edema and alveolar hemorrhage in the non-plasmin group.

There are several limitations to our study. First, to produce thrombus in the donor lungs, we used a model that consisted of 120 minutes of warm ischemia without heparinization, which represents a longer warm ischemic time than the clinical scenario of uncontrolled donation after cardiac death. A thrombosis model without dispersion is difficult to design, whereas the method used here is a simple way to initiate thrombosis in a donor lung. Another limitation with the model used here is that the lungs were ventilated with negative pressure during the EVLP period, whereas, in the clinical setting, positive ventilation is used.

In conclusion, in this clinically simulated study, our results confirm that plasmin administration during EVLP can recondition the donor lung not only during EVLP but also after reperfusion.

Disclosure statement

The authors have no conflicts of interest to disclose.

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ORIGINAL ARTICLE

THORACIC

Winner of the 2014 ESTS - Brompton Prize

Living-donor lobar lung transplantation provides similar survival to cadaveric lung transplantation even for very ill patients[†]

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Abstract

OBJECTIVES: Living-donor lobar lung transplantation (LDLLT) has been performed as a life-saving procedure for critically ill patients who are unlikely to survive the long wait for cadaveric lungs. The purpose of this study was to compare the preoperative condition and outcome of LDLLT patients with those of conventional cadaveric lung transplantation (CLT) patients.

METHODS: A new lung transplant programme was established in 2008 at Kyoto University. Between June 2008 and January 2014, we performed 79 lung transplants, including 42 LDLLTs (10 single, 32 bilateral) and 37 CLTs (22 single, 15 bilateral). Data collected included pre- and perioperative variables and mid-term survival. All data were analysed retrospectively as of January 2014.

RESULTS: The majority of patients were female (57.1%) in the LDLLT group and male (64.9%) in the CLT group. The average age was similar (36.6 ± 20.7 vs 39.7 ± 12.6 years, $P = 0.42$) between the two groups. Preoperatively, interstitial lung disease was more common in LDLLT patients than in CLT patients (47.6 vs 24.3%, $P = 0.048$); prior haematopoietic stem cell transplantation was performed more often in LDLLT patients than in CLT patients (33.3 vs 13.5%, $P = 0.040$) and there were more steroid-dependent LDLLT patients than CLT patients (64.3 vs 29.7%, $P = 0.0022$). Based on preoperative criteria of lower body mass index (17.2 ± 4.0 vs 19.3 ± 3.3 kg/m^2, $P = 0.013$), less ambulatory ability (42.9 vs 86.5%, $P = 0.0001$) and more ventilator dependence (11.9 vs 2.7%, $P = 0.12$), LDLLT patients were more debilitated than CLT patients. LDLLT patients required longer postoperative mechanical ventilation than CLT patients (15.6 ± 16.2 vs 8.5 ± 8.1 days, $P = 0.025$). However, 1- and 3-year survival rates were similar between the two groups (89.7 and 86.1% vs 88.3 and 83.1%, $P = 0.55$). All living donors returned to their previous lifestyles without restriction.

CONCLUSIONS: Although LDLLT patients were in a worse preoperative condition than CLT patients, LDLLT patients demonstrated survival rates similar to CLT patients. LDLLT is a viable option for patients too ill to survive a long waiting time for cadaveric donors.

Keywords: Living-donor lobar lung transplantation • Cadaveric lung transplantation • Interstitial lung disease • Haematopoietic stem cell transplantation

INTRODUCTION

The demand for organs from brain-dead donors for transplantation is ever increasing and far exceeds the supply. It is especially true for lung transplantation because more than half of potential donor lungs are injured and are no longer suitable for transplantation. Living-donor lobar lung transplantation (LDLLT) has been a reported option for critically ill patients since the early 1990s [1]. Although LDLLT was initially performed in the USA, its use has decreased there because of the recent change by the Organ Procurement and Transplantation Network to an urgency/benefit allocation system for cadaveric donor lungs. For the past several years, reports on LDLLT almost exclusively have been from Japan [2-4], where the average waiting time for a cadaveric lung is still more than 2 years.

The outcome of LDLLT varies among transplant centres. The first author and his colleagues in the Okayama University group

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have reported exceptionally good results after LDLLT [2–4]. In 43 patients undergoing LDLLT between 1998 and 2006, the 5-year survival rate was 87.6% with follow-up between 1 and 98 months [4]. During the same period, cadaveric lung transplantation (CLT) was performed in only 9 patients due to the severe scarcity of brain-dead donors. It was not practically possible to compare the two procedures during that period.

The first author moved to Kyoto University where a new lung transplant programme was established in 2008. The Japanese Organ Transplant Law was amended in 2010 and the number of organ donations by brain-dead donors significantly increased [5]. Thus, the comparison of LDLLT and CLT became possible in the newly established programme.

The purpose of this retrospective study was to compare the preoperative condition and outcome of LDLLT patients with those of conventional CLT patients in a single institution.

PATIENTS AND METHODS

In 2008, a new lung transplant programme was approved by the Ethics Committee at Kyoto University Graduate School of Medicine. Following multidisciplinary assessment, each case was carefully discussed by the Lung Transplant Evaluation Committee at Kyoto University Hospital.

Patients requiring CLT from brain-dead donors were registered at the Japan Organ Transplantation Network. The Japanese organ allocation process for lung transplantation has been based mainly on accrued time on a waiting list. Patients being considered for LDLLT must meet the criteria for conventional CLT. The policy of our programme has been to limit LDLLT to critically ill patients who are unlikely to survive the long wait for cadaveric lungs. Our acceptance criteria for living donors included only immediate family members including blood-related relatives within the third degree or a spouse. Size matching criteria in LDLLT have been previously described [2]. LDLLT usually requires two healthy donors who donated either a right or a left lower lobe, but finding two healthy donors was not easy. When only one donor was available with adequate size matching, we performed a single LDLLT.

Perioperative management was essentially the same between LDLLT and CLT patients. Postoperative immunosuppression consisted of triple drug therapy with cyclosporine (CSA) or tacrolimus (FK), and azathioprine or mycophenolate mofetil, and corticosteroids. Induction cytolytic therapy was rarely used. The CSA-based drug combination was chosen for patients with infectious lung diseases, paediatric patients and patients on steroids. The FK-based drug combination was used for other patients. We judged acute rejection on the basis of radiographic and clinical findings without transbronchial lung biopsy.

This study was a retrospective study of 79 consecutive patients who underwent lung transplantation at Kyoto University Hospital between June 2008 and January 2014. These patients were stratified by type of transplant procedure: LDLLT ($n = 42$) and CLT ($n = 37$). The average waiting time for cadaveric donor organs was 761 days. Data collected included pre- and perioperative variables and mid-term survival. All data were analysed retrospectively as of January 2014. This study protocol was approved by the Institutional Review Boards of Kyoto University Hospital.

All values are given as mean ± standard deviation. Bivariate comparison of continuous variables was performed with Student's *t*-test. Associations between categorical variables were tested by the Pearson χ^2 test. Observed survival data were reported as Kaplan–Meier estimates; the log rank test was used to explore the significance of the difference between the two groups. Differences were considered significant at a probability value of less than 0.05. The statistical software used for the analysis was EXCEL STATISTICS 2010 (Social Survey Research Information Co., Ltd, Tokyo, Japan).

RESULTS

Pretransplant variables

Pretransplant patient characteristics are summarized in Table 1. The LDLLT group included 12 children (29%) and 5 adults 60 years of age or older (11.9%). The CLT group included no children and 1 adult (2.7%) 60 years old. However, the average age was similar between the groups (36.6 ± 20.7 vs 39.7 ± 12.6 years, $P = 0.42$).

Because only one or two lobes were implanted into LDLLT recipients, size matching was an important issue. It was for this reason that LDLLT recipients were more often female, shorter in height and lighter in weight than CLT recipients. The LDLLT group had a lower body mass index (BMI) (17.2 ± 4.0 vs 19.3 ± 3.3 kg/m^2, $P = 0.013$) and included more patients with BMI < 17 than the CLT group (50.0 vs 24.3%, $P = 0.019$).

Preoperative diagnosis and conditions

Interstitial lung disease was more common in the LDLLT group than in the CLT group (47.6 vs 24.3%, $P = 0.048$). Emphysema and lymphangioleiomyomatosis were found only in the CLT group. Retransplantation was performed in two LDLLT patients.

Prior allogeneic haematopoietic stem cell transplantation (HSCT) was performed in more LDLLT patients than in CLT patients (33.3 vs 13.5%, $P = 0.040$). The LDLLT group included more steroid-dependent patients than the CLT group (64.3 vs 29.7%, $P = 0.0022$).

The LDLLT group included patients who were less ambulatory than the CLT patients (42.9 vs 86.5%, $P = 0.0001$) and tended to include more ventilator-dependent patients (11.9 vs 2.7%, $P = 0.12$).

Donor characteristics

Among the 74 living donors, 20 were the sons of recipients, 15 were mothers, 8 were daughters, 7 were fathers, 6 were sisters, 6 were wives, 5 were husbands, 5 were brothers, 1 was an uncle and 1 was an aunt. There were 36 male donors and 38 female donors with ages ranging from 20 to 60 years. Among the 37 brain-dead donors, there were 28 male donors and 9 female donors with ages ranging from 21 to 68 years. Female donors were more common among living donors than brain-dead donors (51.2 vs 24.3%, $P = 0.0066$). The average age was similar between the groups (39.6 vs 42.2 years, $P = 0.30$).

Operative variables

Operative variables are summarized in Table 2. Bilateral transplantation was performed more frequently in the LDLLT group than in the CLT group (76.2 vs 40.5%, $P = 0.0013$). All patients (100%) required cardiopulmonary support during LDLLT, but more than half of patients (51.4%) underwent CLT without cardiopulmonary support. The total ischaemic time was significantly

Table 1: Pretransplant patients' characteristics

	Living-donor lobar lung transplantation (n = 42)	Cadaveric lung transplantation (n = 37)	P-value
Age (year)	36.6 ± 20.7	39.7 ± 12.6	0.42
<17	12 (28.6%)	0 (0%)	
17–59	25 (59.5%)	36 (97.3%)	
≥60	5 (11.9%)	1 (2.7%)	
Gender			
Male	18 (42.9%)	24 (64.9%)	0.051
Female	24 (57.1%)	13 (35.1%)	
Height (cm)	148.9 ± 20.3	164.3 ± 8.1	0.0001
Weight (kg)	40.0 ± 16.4	52.1 ± 10.3	0.0003
Body mass index (kg/m^2)	17.2 ± 4.0	19.3 ± 3.3	0.013
Body mass index < 17	21 (50.0%)	9 (24.3%)	0.019
Diagnosis			
Interstitial lung disease	20 (47.6%)	9 (24.3%)	0.048
Bronchiolitis obliterans	13 (31.0%)	5 (13.5%)	0.065
Pulmonary hypertension	5 (11.9%)	2 (5.4%)	0.31
Emphysema	0 (0%)	5 (13.5%)	0.021
Lymphangioleiomyomatosis	0 (0%)	3 (8.1%)	0.060
Retransplantation	2 (4.8%)	0 (0%)	0.18
Others	2 (4.8%)	13 (35.1%)	
Prior haematopoietic stem cell transplantation			
Yes	14 (33.3%)	5 (13.5%)	0.040
No	28 (66.7%)	32 (86.5%)	
Steroid-dependent			
Yes	27 (64.3%)	11 (29.7%)	0.0022
No	15 (35.7%)	26 (70.7%)	
Pretransplant condition			
Ambulatory	18 (42.9%)	32 (86.5%)	0.0001
Ventilator-dependent	5 (11.9%)	1 (2.7%)	0.12

Table 2: Operative variables

	Living-donor lobar lung transplantation (n = 42)	Cadaveric lung transplantation (n = 37)	P-value
Type of transplant			
Bilateral	32 (76.2%)	15 (40.5%)	
Single	10 (23.8%)	22 (59.5%)	0.0013
Use of cardiopulmonary support			
Yes	42 (100%)	18 (48.6%)	<0.0001
No	0 (0%)	19 (51.4%)	
Total ischaemic time (min)	161 ± 47	459 ± 95	<0.0001

shorter in the LDLLT group than in the CLT group (161 ± 47 vs 459 ± 95 min, P < 0.0001).

Early post-transplant outcome

The PaO_2/FiO_2 ratio was significantly better in the LDLLT group than in the CLT group immediately after reperfusion (434 ± 121 vs 303 ± 117, P < 0.0001). The frequency of tracheostomy and extracorporeal membrane oxygenation support was similar between the two procedures; however, the duration of mechanical ventilation required was significantly longer after LDLLT than after CLT (15.6 ± 16.2 vs 8.5 ± 8.1 days, P = 0.025) (Table 3).

The CSA-based drug combination was used more commonly after LDLLT (57.1%), and the FK-based drug combination was used more commonly after CLT (59.5%). The incidence of steroid pulse use for suspected acute rejection was slightly higher after LDLLT than after CLT (0.9 ± 0.7 vs 0.6 ± 0.7, P = 0.11).

Thirty-day mortality occurred in only 1 patient (2.4%) after LDLLT. Hospital mortality was encountered in 3 patients (7.1%) after LDLLT and 2 patients (5.4%) after CLT.

Survival and causes of death

At the time of final data analysis on 31 January 2014, the mean time from LDLLT to final analysis for the 42 recipients was 28 months (range 2–69 months). The mean time from CLT to final analysis for the 37 recipients was 17 months (range 1–42 months). Survival curves are shown in Fig. 1. One- and 3-year survival rates were similar between the LDLLT and CLT groups (89.7 and 86.1% vs 88.3 and 83.1%, P = 0.55).

Causes of death are summarized in Table 4. There were six deaths after LDLLT and five deaths after CLT during the observation period. Chronic lung allograft dysfunction (CLAD) was the cause of death in 2 patients in each group.

Donor outcome

We encountered minor surgical morbidities such as prolonged air leak, wound infection and pleural effusion requiring chest tube

Table 3: Early post-transplant outcome

	Living-donor lobar lung transplantation (n = 42)	Cadaveric lung transplantation (n = 37)	P-value
PaO_2/FiO_2 ratio immediately after reperfusion	434 ± 121	303 ± 117	<0.0001
Tracheostomy			
Yes	24 (57.1%)	17 (45.9%)	0.32
No	18 (42.9%)	20 (54.1%)	
Use of ECMO support			
Yes	6 (14.3%)	7 (18.9%)	0.58
No	36 (85.7%)	30 (81.1%)	
Duration of mechanical ventilation	15.6 ± 16.2	8.5 ± 8.1	0.025
Immunosuppressant			
CSA + AZA or MMF + P	24 (57.1%)	15 (40.5%)	0.050
FK + AZA or MMF + P	14 (33.3%)	22 (59.5%)	
Other	4 (9.5%)	0 (0%)	
Steroid pulse for acute rejection <30 days	0.9 ± 0.7	0.6 ± 0.7	0.11
Thirty-day mortality	1 (2.4%)	0 (0%)	0.34
Hospital mortality	3 (7.1%)	2 (5.4%)	0.75

ECMO: extracorporeal membrane oxygenation; CSA: cyclosporine; AZA: azathioprine; MMF: mycophenolate mofetil; P: prednisone; FK: tacrolimus.

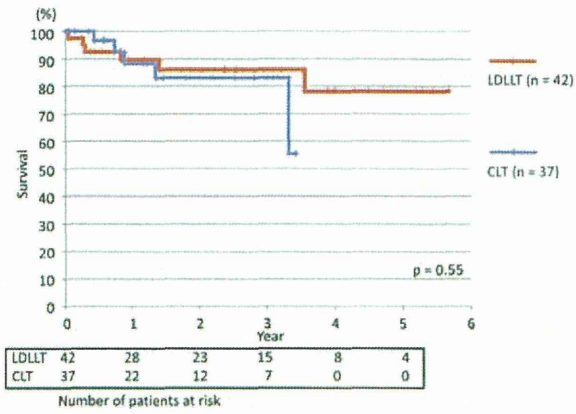

Figure 1: Actuarial survival after living-donor lobar lung transplantation (LDLLT, n = 42) compared with that after cadaveric lung transplantation (CLT, n = 37). One- and 3-year survival rates were similar between the two groups (89.7 and 86.1% for LDLLT vs 88.3 and 83.1% for CLT, P = 0.55).

placement after donor lobectomy. However, all living donors returned to their previous lifestyles without restriction. Vital capacity recovered up to 90% of the preoperative value at 1 year after donation.

DISCUSSION

LDLLT was developed by the group of University of Southern California (USC) in order to mitigate the growing competition for brain-dead donor lungs [1]. Because of possible serious complications in the donor lobectomy, LDLLT is indicated only for critically ill patients who are unlikely to survive the long wait for cadaveric lungs. However, if the recipient is too ill, lobectomies from healthy donors would not be justifiable. LDLLT can be justified only when it will provide an equal or better outcome than conventional CLT. In Japan, LDLLT was the only realistic option for most patients until 2010 when the Japanese Organ Transplant Law was amended [2–4]. The revision of the law significantly increased the number of organ donations by brain-dead donors and CLT has become a more realistic option for adult Japanese patients since then [5]. There are nine lung transplant centres in Japan and seven of them are currently active. Only about 50 brain-dead donors have been available annually in the whole country even under the revised transplant law, which accounts for only 0.4 donors per million of the population. The lung recovery rate is more than 60% because we accept marginal donors as much as possible to maximize the number of CLTs. Sixty lung transplantations were conducted throughout Japan in 2013, including 20 LDLLTs and 40 CLTs.

The average waiting time for CLT still exceeds 2 years in Japan and the mortality rate on the waiting list is 39%. The algorithm for brain-dead donor lung allocation is based primarily on accrued time on the waiting list, a situation which favours patients with slowly progressive diseases and disadvantages patients with rapidly progressive diseases. As a result, patients with emphysema and lymphangioleiomyomatosis do not receive LDLLT because they can survive a long waiting time to receive CLT. On the other hand, interstitial lung disease, including idiopathic pulmonary fibrosis (IPF), is more common in LDLLT patients than in CLT patients. Of note, the International Association for Heart and Lung Transplantation reported that IPF is one of the risk factors for 1-year mortality after adult CLT [6].

In the present study, one-third of LDLLT patients (n = 14) suffered from chronic progressive pulmonary complications after HSCT. These patients are commonly believed to be high-risk candidates for lung transplantation because long-term pretransplant intake of immunosuppressant drugs often causes organ dysfunction of other organs and increases the risk of various infections [7, 8]. Another concern is the possible recurrence of haematological malignancy [9]. It is for these reasons that most transplant centres would not have accepted these patients.

The higher prevalence of steroid-dependent patients in the LDLLT group than in the CLT group was the result of the higher prevalence of interstitial lung disease and pulmonary complications after HSCT in LDLLT patients. Chronic steroid use prior to transplantation has been reported to be a risk factor for mortality in CLT [6]. Contraindications to CLT include current high-dose systemic corticosteroid therapy because it may increase airway

Table 4: Causes of death

	Pt. no.	Age, gender	Diagnosis	Procedure	Timing of death	Cause of death
LDLLT						
	1	11 years, M	IP after HSCT	Right single	14 days	Sepsis
	2	64 years, M	IPF	Bilateral	3 months	Aspiration pneumonia
	3	12 years, F	Retransplantation	Left single	3 months	Primary graft dysfunction
	4	57 years, F	BO after HSCT	Bilateral	5 months	CLAD
	5	60 years, M	IPF	Bilateral	10 months	PTLD
	6	44 years, M	BO after HSCT	Bilateral	43 months	CLAD
CLT						
	1	44 years, M	BO after HSCT	Right single	5 months	PTLD
	2	29 years, M	BO after HSCT	Right single	7 months	CLAD
	3	48 years, M	Emphysema	Right single	11 months	Aspergillosis
	4	40 years, F	LAM	Bilateral	16 months	Sepsis
	5	50 years, M	Emphysema	Left single	40 months	CLAD

Pt. no.: patient number; LDLLT: living-donor lobar lung transplantation; CLT: cadaveric lung transplantation; IP: interstitial pneumonia; HSCT: haematopoietic stem cell transplantation; IPF: idiopathic pulmonary fibrosis; BO: bronchiolitis obliterans; LAM: lymphangioleiomyomatosis; CLAD: chronic lung allograft dysfunction; PTLD: post-transplant lymphoproliferative disorder; M: male; F: female.

complications, although low-dose pretransplantation corticosteroid therapy is acceptable. We have accepted the use of high-dose systemic corticosteroid therapy in LDLLT patients. Excellent bronchial healing was observed in 70 of 74 (95%) anastomoses. Various other factors, such as short donor bronchial length, high blood flow in the implanted small grafts and well-preserved lung parenchyma with short ischaemic times, may contribute to better oxygen supply to the donor bronchus, thus resulting in excellent bronchial healing in LDLLT patients [10].

It was obvious that the pretransplant condition was worse in LDLLT patients than in CLT patients. Half of the LDLLT patients had a BMI less than 17 kg/m^2. It has been well documented that malnourished patients have increased morbidity and mortality after lung transplantation [11, 12]. In our LDLLT experience, all patients were oxygen-dependent and less than half of the patients were able to walk. Use of LDLLT in patients already on a ventilator is controversial. We successfully performed LDLLT for all 5 patients who had been on a ventilator for as long as 10 months [13]. The USC group reported that patients on a ventilator preoperatively had significantly worse outcomes among their 123 LDLLTs [1].

Two patients received a retransplantation in the LDLLT group. The first patient was a 12-year-old female with pulmonary hypertension and repaired transposition of the great arteries. She underwent a right single-lobe transplantation from her father and required a left single-lobe transplantation as a retransplantation from her mother on day 17 because of progressive graft dysfunction. Massive bleeding without a detectable cause occurred at the time of retransplantation and she died of multiple organ failure on day 108. The second patient was a 34-year-old female with IPF undergoing initial bilateral LDLLT from her husband and her mother. The graft function was lost due to antibody-mediated rejection and bronchiolitis obliterans. She received a successful rebilateral LDLLT from her brother and her sister 34 months after the initial bilateral LDLLT. Twenty-one months after the rebilateral LDLLT, she returned to a normal lifestyle.

The ischaemic time for the LDLLT group was much shorter than for the CLT group because essentially no transportation time was required. Although only one or two lobes were transplanted and it always was performed under cardiopulmonary support, LDLLT provided significantly better oxygenation than CLT immediately after reperfusion. We believe that using a 'small but perfect graft' is a great advantage in LDLLT.

Meticulous postoperative management is mandatory after lung transplantation. Because the LDLLT recipients were sicker and more cachexic, the average duration of mechanical ventilator after transplantation was over 2 weeks and tracheostomy was required in more than half of the recipients in spite of better oxygenation immediately after reperfusion. A similar or higher incidence of steroid pulse use for suspected acute rejection after LDLLT than after CLT could be explained by our strategy to judge acute rejection on the basis of radiographic and clinical findings without transbronchial lung biopsy. In spite of the stormy postoperative course after LDLLT, the hospital mortality rate was 7.1%, which was similar to CLT (5.4%).

CLAD including bronchiolitis obliterans syndrome (BOS) has been the major obstacle after CLT [6, 14]. Theoretically, human leucocyte antigen (HLA) similarity in cases of blood-related donation may be beneficial, but the role of HLA mismatches may be more complicated when two different donor lobes are implanted into one recipient in bilateral LDLLT [1]. Transplanting two lobes obtained from two different donors appears to be beneficial in the long term because the contralateral unaffected lung may function as a reservoir in case of unilateral BOS [15]. BOS occurred in 8 of 42 recipients (19%) in the LDLLT group and 6 of 37 recipients (16%) in the CLT group. The question remains whether patients receiving LDLLT will have less CLAD than those receiving CLT. The observation period in this study was too short to answer this question.

The survival rate after LDLLT varies among transplant centres. There are only three groups that have reported a summary of recipient outcomes. The USC group published their 10-year experience on 123 LDLLT recipients including 39 children [1]. One, 3- and 5-year survival rates were 70, 54 and 45%, respectively. The St Louis group reported similar results in 38 paediatric LDLLT recipients [16]. The first author and his colleagues of the Okayama University group have reported on 43 LDLLTs with a 5-year survival rate of 87.6% during follow-up between 1 and 98 months. In the current study, 1- and 3-year survival rates were 89.7 and 86.1%, respectively, after LDLLT in an observation period of 2–69 months. The current study demonstrated that good results after LDLLT are reproducible.

Successful LDLLT largely depends on donor outcome. Relatively high donor morbidity after lobectomy has been described in previous reports [17]. In our experience, all donors returned to their previous lifestyles without any restrictions. However, we found possible postoperative deterioration in several aspects of health-related quality of life and dyspnoea in our prospective study of multidimensional evaluation [18]. Potential donors should be competent, willing to donate free of coercion, medically and psychosocially suitable, fully informed of the risks and benefits as a donor, and fully informed of risks, benefits and alternative treatment available to the recipient. In our institution, potential donors are interviewed at least three times to provide them multiple opportunities to question, reconsider or withdraw as a donor.

It is clear that the LDLLT group included more patients with high-risk factors such as interstitial lung disease, pulmonary complications after HSCT, steroid dependency, malnutrition, bed-bound condition and ventilator dependency than the CLT group. Although recipients' preoperative conditions were worse, LDLLT provided similar survival to CLT patients.

CONCLUSION

We conclude that LDLLT is a viable option for patients too ill to survive a long waiting period for cadaveric donors.

Limitation

Limitations of the present study included that it is a non-randomized retrospective study, the small number of transplant procedures and the relatively short duration of patient follow-up.

Conflict of interest: none declared.

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APPENDIX. CONFERENCE DISCUSSION

Dr P.-E. Falcoz (Strasbourg, France): First, I want to present apologies from Pascal Thomas who was effectively not able to attend this meeting due to a sudden and recurrent strike in France, so yesterday he sent me his comments concerning your paper, and I will read them now.

Dr Date, your study clearly demonstrates that living-donor lobar lung transplantation offered the possibility of transplantation to a group of patients who would otherwise probably have died. Moreover, the results, albeit in a very sick group of recipients, were at least as good as you and many others could achieve with lung from cadaveric donors, and last but not least, the prospect of reduced chronic rejection seems realized.

Pioneering US groups failed to report such good long-term outcomes. Those initial disappointing results combined in 2005 to encourage the adoption of the LAS, the lung allocation scoring system, and probably may explain why the need for living-donor lobar lung transplantation decreased in the US. Similarly in France, a proactive national policy regarding organ donation was conducted 10 years ago. That aimed at doubling lung graft availability from cadaveric donors. Furthermore, in France a so-called "high emergency" pathway was created in 2007 that contributed to speeding up the organ offer for the sicker patients. Both events lessened the need for living-donor lobar lung transplantation in France.

I have four questions for you that I will ask sequentially. First, to help us to better understand the current status of lung transplantation in Japan, can you elaborate a little bit more on your current national organization and performance, especially since 2010, and the changes in the law that you mentioned: how many active transplant centres are there in Japan, what is the overall number of diseased organ donors per median population, the lung recovery rate among cadaveric donors, and the number of lung transplants per million population?

Dr Date: We have nine certified lung transplant centres in Japan and seven of them are active. I am ashamed to tell you that only about 50 brain-dead donors are available annually in Japan, which means about 0.4 per million population. It's a very small number.

Dr Falcoz: Second question. Even if it was not a real part of the objectives of your present study, can you give us some more information on surgical morbidity and late functional loss in the living donors?

Dr Date: Yes. The outcome of the living donor is as important as that of the recipient. We have encountered some minor surgical morbidities such as prolonged air leakage, early infection, and pleural effusion requiring chest tube

insertion. However, they all recovered and returned to their normal lives. Regarding pulmonary function after donor lobectomy, vital capacity is lost permanently, otherwise it recovered up to about 90% at one year.

Dr Falcoz: As you mentioned, size matching between donor and recipients may be an important technical issue in living donor transplantation. On the other hand, wide discrepancies in lung sizing have been reported not to affect overall post-transplant survival or pulmonary function. Therefore, a greater degree of lung size mismatch can likely be accepted, thereby improving patient odds of undergoing transplantation. Can you give us some information on your personal experience and point of view on the mismatch?

Dr Date: That is also a very important issue. A wide variety of size mismatching does exist in living-donor lobar lung transplantation. However, precise size matching can be done, because you can measure the preoperative pulmonary function of the donors, which cannot be done in cadaveric lung transplantation, and also we do a 3D CT volumetric evaluation both for the recipient and the donor in living-donor lobar lung transplantation.

For an undersized graft, sometimes we preserve an upper lobe of the recipient in a living-donor lobar lung transplant, and for the oversized graft we use a delayed chest closure technique and a contralateral pneumonectomy technique, and sometimes we do volume reduction surgery in the graft using a segmentectomy.

Dr Falcoz: Finally, you showed us excellent long-term survival figures following either cadaveric or living donors. Can you elaborate more on crude and comparative functional results?

Dr Date: The postoperative pulmonary function after living-donor lobar lung transplantation largely depends on the graft size. The average vital capacity at one year was about 70% of the predicted value, and FEV1 also was about 70% of the predicted value, indicating there was no obstructive change in the small graft transplanted in a huge chest cavity.

Dr W. Weder (Zurich, Switzerland): We have to congratulate you on your contribution to living-related lung transplantation. You concluded in your presentation that it's a viable option compared to cadaveric donor transplantation. Let's say you had 10 times more cadaveric lungs in Japan, would you come to the same conclusion?

Dr Date: I think if we had 10 times more, probably the need for living-donor lobar lung transplantation would be much smaller, and the last conclusion would be the same, but in very, very selected patients.

Dr Weder: Like who?

Dr Date: Like the patient who is dying very quickly.

Dr D. Van Raemdonck (Leuven, Belgium): You have shown similar survival between the two groups; however, the living-lobar recipients were much sicker, so you would expect a worse survival. There was also a difference in the type of transplant, single versus bilateral, between both groups. Do you think this has had an impact on the survival results? In other words, do you think in the long-term results, the cadaveric recipients become worse because of bronchiolitis obliterans?

Dr Date: That is a very good question. Bilateral transplantation is better than single lung transplantation regardless of the type of procedure; cadaveric living bilateral is better than single. But in living-donor lobar lung transplantation, it depends on the availability of the donor in the family. If the patient is small enough and if they find only one single donor, we accept that, knowing that the result is worse than the bilateral procedure. That occurred in about 25% of our experience.

In cadaveric lung transplantation, I know you like the bilateral procedure much more than single, but we accept more singles, because of the shortage of donor organs, to maximize the number of cadaveric lung transplantations. As to the question of whether there may be a difference in the longer term results, we don't know yet.

Dr G. Leschber (Berlin, Germany): You showed the different gender of the recipients and that you had many more women receiving living-donor transplants. What about the donors? You didn't show us the sex of the donors. I suppose you have many more women giving their lungs, especially when you look at the group of the patients and the ages of these patients, because you showed us that there were many children. Can you comment on this?

Dr Date: That is also an important question. Regarding the donors, there are more female living-donor lobar lung transplants and more males in the cadaveric lung transplants, a significant difference, and the age was similar between the two groups.

Dr Leschber: You said there are more. Do you know the percentage?

Dr Date: About 60% of the living donors were female and about 70% of the cadaveric donors were male.

Dr I. Inci (Zurich, Switzerland): You mentioned that the living-donor lobar lung transplant recipients were sicker. Do you accept patients with extracorporeal life support? So were they on preoperative extracorporeal life support?

Dr Date: We have not accepted a patient on ECMO or any other modality of cardiopulmonary support yet. We may accept them if the patient is already in our hospital and deteriorating quickly, and then we put the ECMO in and after full evaluation we might probably accept such a patient, but it has not happened yet.

Dr I. Bravio (Lisbon, Portugal): I would like to ask you two questions. You transplanted sick patients with living donors and then you showed that you had two patients that needed to be re-transplanted. What I wanted to know is what were the reasons for the re-transplantation and at what time interval, and in these patients which lung did you use for re-transplantation and did these have worse outcomes or not?

Dr Date: We have done two patients as a re-transplantation. The first patient, whom I probably should not have done, was a 12-year-old female who had a repair of a great vessel malformation for hypertension, but she had persistent pulmonary hypertension. We did a right single-lung transplantation for this patient from the father, but the graft was too small. So we did a left single-lung transplantation from the mother. That occurred about two weeks after the first operation. During the second lobar lung transplantation, significant bleeding in the bronchus occurred without a clear cause, and we lost that patient.

The second patient received a bilateral living-donor lobar lung transplant, again from the family, different donors, about two years after the initial operation, and it went very well, and she is doing very well two years after re-transplantation.

Case Reports

Cardiac Allograft Vasculopathy Can Be Distinguished From Donor-Transmitted Coronary Atherosclerosis by Optical Coherence Tomography Imaging in a Heart Transplantation Recipient

Double Layered Intimal Thickness

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SUMMARY

Although survival after heart transplantation (HTx) has improved in recent years, cardiac allograft vasculopathy (CAV) is still the leading cause of remote morbidity and mortality in HTx recipients, partly because of difficulty with its diagnosis. In general, routine surveillance for CAV is advocated with coronary angiography accompanied by intravascular ultrasound (IVUS) if necessary. However, these modalities have limitations with respect to low spatial resolution, and sufficient qualitative/quantitative assessment of coronary intima has not been accomplished. Recently, optical coherence tomography (OCT) has emerged as a novel intracoronary imaging technique using an optical analogue of ultrasound with a spatial resolution of 10-20 μm, which is 10 times greater than IVUS. We here experienced a 49-year-old male who received a HTx 3 years ago, and OCT was executed during low molecular weight dextran injection. OCT demonstrated distinct double intimal layers probably consisting of a donor-transmitted atherosclerotic layer and an inner intimal proliferation due to CAV, which was indistinguishable by IVUS and virtual histological analyses. We believe that OCT imaging is not only a new loadstar during treatment of CAV but also a new generation modality for screening for early CAV in HTx recipients. (Int Heart J 2014; 55: 178-180)

Key words: Intima, Everolimus, Intravascular ultrasound

Although major improvements have been made in surgical techniques and treatments for acute rejection, accelerated cardiac allograft vasculopathy (CAV) still limits the remote survival in heart transplantation (HTx) recipients.[1)] CAV is a pathologically multifaceted disorder that affects epicardial coronary arteries with different types of lesions including intimal fibromuscular hyperplasia, atherosclerosis, and inflammation.[2)] In distinction from general coronary atherosclerosis, which is marked by focal and eccentric fibrofatty atheroma, CAV involves the entire coronary vasculature diffusely with marked intimal proliferation and concentric vascular thickening and fibrosis.[3)] Typically, HTx recipients do not experience angina because of perioperative denervation, but eventually present with left ventricular dysfunction as a consequence of progressed myocardial ischemia.[4)] Therefore, an International Society for Heart and Lung Transplantation (ISHLT) working group recommended regular surveys with coronary angiography regardless of the recipient's symptoms for early detection of CAV, accompanied by subsequent IVUS when CAV is suspected.[5)]

Recently, optical coherence tomography (OCT) has emerged as a new generation catheter-based modality that acquires images at a spatial resolution of 10-20 μm, enabling visualization of blood vessel wall microstructure in vivo at an unprecedented level of detail.[6)] However, little is known about adaptation of OCT for analyses of CAV. Hence, we experienced a chance to conduct OCT along with coronary angiography and IVUS in a heart transplantation (HTx) recipient, and discuss the utility of OCT.

CASE REPORT

In 2010, a 46-year-old male with dilated cardiomyopathy received a HTx from a male adult donor after undergoing 2 years of left ventricular assist device support. His postoperative course was uneventful under prescription of tacrolimus, mycophenolate mophetil, prednisolone, and 2.5 mg/day of rosuvas-

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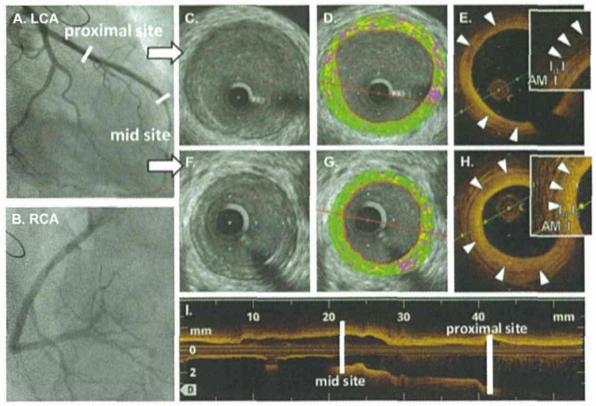

Figure. Coronary angiography images of left coronary artery (**A**) and right coronary artery (**B**); intravascular ultrasound images of the proximal (**C**) and the mid (**F**) sites of LAD; iMAP images of the proximal (**D**) and mid (**G**) sites of LAD; optimal coherence tomography images of the proximal (**E**) and mid (**H**) sites of LAD. Figure 1I shows longitudinal OCT image of LAD. A indicates adventitia; M, media; I, intima; I_d, donor-transmitted atherosclerosis; and I_c, intimal proliferation due to CAV. Arrowheads represent borderline between donor-transmitted atherosclerosis layer and intimal proliferation layer due to CAV.

tatin, except for 1 instance of cellular rejection with ISHLT grade 3A at 3 weeks after HTx, which was treated by steroid pulse therapy. At 3 months after HTx, diffuse slight plaque at a mid to proximal site of the left anterior descending coronary artery (LAD) was observed by intravascular ultrasound (IVUS). After detection of diffuse mild plaque at a mid to proximal site of the LAD by IVUS at 6 months after HTx, mycophenolate mophetil was switched to everolimus. Prednisolone was tapered off in August 2012.

In August 2013, he was admitted to our hospital for regular follow-up. His height and weight were 180 cm and 66 kg. His plasma B-type natriuretic peptide concentration was 56.4 pg/mL, and his serum creatinine concentration was 1.12 mg/dL on admission. Trough concentrations of tacrolimus and everolimus were 6.7 and 3.9 ng/mL, respectively. His ejection fraction on transthoracic echocardiography by Simpson's biplane method was 67% with a left ventricular end-diastolic diameter of 46 mm. Cytomegalovirus antigenemia and %panel reactive activity were assayed but both were negative.

According to a hemodynamic study and endomyocardial biopsy, his intracardiac pressure was normal together with no cellular rejection (ISHLT grade 0) and no complement deposition. Coronary angiography indicated no significant stenosis in any coronary artery (Figure 1A and B). IVUS images were recorded (iLab™, Boston Scientific, Corporation, Natick MA) from the mid portion to the left main coronary artery with an automated pullback system at a speed of 0.5 mm/s, using a 2.5F, 40-MHz IVUS catheter (Atlantis™ SR Pro, Boston Scientific Corporation, Natick, MA, USA). Analyzed IVUS images showed diffuse concentric plaque at a mid to proximal site of the LAD (maximal %plaque area was 38.4% at the mid site) (Figures 1C and F). iMAP images (Boston Scientific Corporation) revealed histological tissue characterization of the LAD, and over 70% of the intimal area was occupied with fibrotic component (Figures 1D and G). Subsequently, the IVUS catheter was replaced with a 2.7F OCT catheter (C8 DragonFly™ JP, St Jude Medical, St Paul, MN, USA). During low molecular weight dextran injection for the clearance of blood (30 mL at 4 mL/s by power injection), OCT images (C8-XR™ system, ILUMIEN™ OPTIS™ Imaging system, St Jude Medical) were recorded from the mid to proximal portion of the LAD at an automatic pull-back speed of 20 mm/s and a frame rate of 100/s. Three layers of components consisting of intima, media, and adventitia were observed separately. Moreover, double homogenous intimal layers that were separated by a thin threshold line were observed (Figures 1E, H, and I).

Discussion

Considering the future of coronary angiography that visualizes only the coronary lumen and CAV that facilitates diffuse and concentric proliferation of intima, an early diagnosis of CAV only by coronary angiography is sometimes difficult. In contrast, IVUS can quantify coronary plaque, and some recent investigators recommend IVUS for routine surveillance of CAV.[7] Consistently, although there appeared to be no angiographic stenosis in the LAD in the present case (Figure 1A and B), mild plaque was detected at a mid to proximal site of the LAD by IVUS analyses (Figure 1D and E). However, intimal thickening is only indirectly evaluated as the intima-media thickening by IVUS because the boundary of intima and media cannot be distinguished by this method as shown also in the present case.[8] OCT is a new imaging procedure with a spatial resolution of approximately 10-20 μm, which is 10-fold greater than that of IVUS.[6] As shown in Figures 1E and H, OCT could obviously identify the layer of media as a lower-echoic line, which could not be identified by IVUS. When assessing the quality of an intracoronary structure accurately, OCT seems to have more potential than IVUS.[9]

Although few reports have performed OCT analyses for CAV,[10,11] Cassar, *et al* introduced "layered complex plaque" as one of the advanced types of CAV. They speculated that such multi-layer patterns consisting of multi-components within intimal thickening may be a pathological hallmark of repeatedly healed intimal erosions through progression of CAV.[12] We could detect a clear and pronounced boundary line within the intimal layer at the mid to proximal site in the LAD, but both layers seemed homogeneous. Consistently, iMAP images, which are obtained by using a pattern recognition algorithm on the spectra obtained from a fast Fourier transformation and histology-derived database,[13] could not distinguish between the 2 intimal layers histologically. Both layers mainly consisted of a fibrotic component. Soon after HTx, we observed only slight diffuse plaque at the mid to proximal site in the LAD by IVUS imaging, which must have been donor-transmitted atherosclerosis. Considering these results, there may be 2 mono-component intimal layers consisting of donor-transmitted atherosclerosis (shown as "I_d" in Figure 1E and H) and successive inner intimal proliferation due to CAV after HTx (shown as "I_c" in Figure 1E and H), which could not be distinguished by conventional modalities other than OCT. Repeated observation of the inner layer by OCT imaging would strengthen this double layer hypothesis.

Statins,[14] vasodilators,[15] and immunosuppressive agents such as mycophenolate mofetil[16] and everolimus[17] are used to treat CAV. However, there have been no standardized loadstars

for the treatment of CAV thus far. Quantitational assay of intimal thickening purely due to CAV separated from donor-transmitted atherosclerosis by OCT imaging would become a key loadstar for assessing the effectiveness of a specific treatment against CAV. Moreover, by distinguishing newly developed CAV from donor-transmitted atherosclerosis, an early and accurate diagnosis of CAV may be feasible.

Should OCT be recommended for all HTx recipients as a screening procedure? Different from IVUS, OCT imaging requires displacement of red blood cells from the vessel lumen during the procedure, and this is generally accomplished by using radiographic contrast.[18] However, there is concern that injection of radiographic contrast would worsen renal dysfunction because HTx recipients often have higher levels of serum creatinine due to daily administration of immunosuppressive agents, like in the present patient.[19] We adopted here low molecular weight dextran instead of radiographic contrast, as recommended by Frick, *et al.*[20] The quality of OCT images was equally high and sufficient to be analyzed compared with those with conventional radiographic contrast. We would like to emphasize that OCT imaging during low molecular weight dextran injection may be a novel new generation procedure for routine surveillance of CAV in HTx recipients.

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Recipients With Shorter Cardiopulmonary Bypass Time Achieve Improvement of Parasympathetic Reinnervation Within 6 Months After Heart Transplantation

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SUMMARY

Although cross-sectional late-phase reinnervation in heart transplantation (HTx) recipients has been demonstrated by several earlier studies, early-phase successive analyses especially for parasympathetic reinnervation remain unknown. Successive heart rate variability (HRV) data calculated by the MemCalc power spectral density method were obtained from 16 non-rejection recipients 1-24 weeks after HTx. High frequency (HF) level representing parasympathetic magnitude increased significantly at 6 months after HTx (from 0.9 ± 0.7 to 4.1 ± 2.8 ms^{2*}). Only intraoperative shorter cardiopulmonary bypass time (181 ± 59 minutes) correlated with a higher level of HF at post-HTx 6 months among all baseline variables (r = -0.530*). Higher level of HF was associated with recovery of tachycardia at post-HTx 6 months (r = -0.514*). In conclusion, parasympathetic reinnervation emerges along with recovery of tachycardia < 6 months after HTx, which is accelerated by shorter intraoperative cardiopulmonary bypass time (*$P < 0.05$ for all). (Int Heart J 2014; 55: 440-444)

Key words: Sympathetic, Heart rate variability, Memcalc, Donor

Heart transplantation (HTx) is the ultimate treatment for patients with stage D heart failure because the deteriorated heart of the recipient is completely replaced with a healthy donor heart. However, complete allograft denervation occurs during the operation,[1)] which results in adverse clinical effects including higher heart rates (HR) at rest, slow acceleration of HR during exercise, decreased exercise tolerability, and absence of angina at coronary ischemia.[2-6)] Recent clinical and experimental studies have provided evidence of progressive partial sympathetic reinnervation during several years after HTx through HR variability (HRV) analyses, positron emission tomography imaging with the catecholamine analogue C-11 hydroxyephedrine, or hormonal measurement.[3,7-12)]

However, most studies were executed by cross-sectional observation at several years after HTx, while fewer studies were conducted successively within postoperative 1 year. Moreover, little has been investigated about parasympathetic reinnervation. We here analyzed successive HRV parameters 1-24 weeks after HTx to investigate postoperative early-stage parasympathetic reinnervation.

METHODS

Patients selection: Sixteen recipients who had received HTx and been followed > 6 months without acute rejection or heart failure at the University of Tokyo Hospital between April 2013 and March 2014 were retrospectively enrolled in the present study (early-stage group). All recipients had been treated with a ventricular assist device (VAD) before HTx, and had undergone a standard HTx procedure with a modified bicaval anastomosis technique.[13)] Rejection was monitored by serial endomyocardial biopsy and hemodynamic studies every 1 week until 1 month, every 2 weeks until 3 months, and then every month until 6 months. All candidates had sinus rhythm during the study period. Written informed consent was obtained before the study enrollment from the patients and/or their family members in all cases. The study protocol was approved by the Ethics Committee of the Graduate School of Medicine, the University of Tokyo [application number 779 (1)].

Immunosuppressant protocol: All recipients were treated with standard triple immunosuppressant therapy including carcineurin inhibitors (cyclosporine or tacrolimus), mycophenolate mophetil, and low dose prednisolone as we previously described.[14-16)] The target trough concentration of cyclosporine was 300-400 ng/mL during the first 3 months, and then re-

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duced to 250-300 ng/mL until 6 months. The trough level of tacrolimus was maintained at 10-15 ng/mL during the first 3 months, and about 10 ng/mL thereafter. Mycophenolate mophetil was initiated within the first 3 days and maintained at a dose of 1500-2000 mg/day. Prednisolone was administered at 1 mg/kg initially, and then tapered off gradually until the first year if possible.

Variables evaluated: Demographic data before HTx such as VAD selection or duration of VAD therapy were obtained. Perioperative and donor data such as ischemic time of donor heart and donor age were also obtained. Laboratory, echocardiographic, and hemodynamic data at 1 week (baseline) and 6 months after HTx were obtained.

HRV spectral analysis: Mean HR and HRV were measured for 5 minutes at 9:00-12:00 AM after 15 minutes of rest in the supine position under fixed 0.25 Hz of respiratory rate along with fasting every 1 week until 1 month, every 2 weeks until 3 months, and every month until 6 months after HTx. Electrocardiographic signals from bipolar leads were transformed to digital signals to calculate the R-R intervals at a sampling rate of 512 Hz. Power spectral analysis of HRV was performed by the MemCalc power spectral density method using a commercial software package (MemCalc/Win, Suwa Trust) that used the maximum entropy method for spectral analysis and the nonlinear least-squares method for fitting analysis.[17] Low frequency (LF) was defined as 0.04 to 0.15 Hz, and high frequency (HF) as 0.15 to 0.40 Hz. The HF power denotes solely the parasympathetic activity, whereas the LF/HF power represents sympathetic activity.

Statistical analysis: All statistical analyses were performed using PASW Statistics 18 (SPSS Inc, Chicago, IL, USA). Categorical variables were summarized as frequencies and percentages, and compared using the Chi-square test or Fisher's exact test as appropriate. Continuous variables are presented as the mean ± standard deviation unless otherwise specified, and compared using the unpaired *t*-test or Mann-Whitney test as appropriate. Pearson's product-moment correlation coefficients were calculated to assess the relationship between HF values at 24 weeks and background parameters. Each HRV parameter was compared with that of baseline by the ad-hoc Dunnett test when repeated analysis of variance was proved to be significant. All hypothesis tests reported were two-tailed, and used a P value < 0.05 as significant.

Results

Baseline characteristics (Table I): All patients were treated by VAD treatment for > 1 year (average, 904 ± 233 days, median, 919 days) before HTx due to dilated cardiomyopathy (14 patients), ischemic cardiomyopathy (1 patient), and dilated phase of hypertrophic cardiomyopathy (1 patient). Mean donor age was 38 ± 14 years (range, 17-60 years-old) and there were 5 males (31%). Mean duration of allograft ischemia was 253 ± 33 minutes (range, 201-298 minutes), and mean cardiopulmonary bypass time was 181 minutes (range, 119-360 minutes). HTx procedures were executed without any complications in all recipients.

Mean recipient age was 36 ± 14 years-old (range, 21-61 years-old) and 9 patients (56%) were male. Six patients (38%) received cyclosporine, and tacrolimus was prescribed for 10

Table I. Baseline Parameters in HTx Recipients

Variables	
Donor parameters	
Age, years	38 ± 14
Male, *n* (%)	5 (31)
Transplant Surgery	
Duration of allograft ischemia, min	253 ± 33
Cardiopulmonary bypass time, min	181 ± 59
Aortic cross-clamp time, min	104 ± 14
Recipients' pre-HTx parameters	
PF LVAD, *n* (%)	8 (50)
CF LVAD, *n* (%)	8 (50)
Duration of VAD treatment, days	904 ± 233
Etiology of ischemia, *n* (%)	1 (6)
Recipients' demographic parameters	
Age, years	36 ± 14
Male, *n* (%)	9 (56)
Body mass index	19.9 ± 2.8
Systolic blood pressure, mmHg	124 ± 24
Diastolic blood pressure, mmHg	68 ± 12
HbA_{1c} (NGSP), %	5.2 ± 0.5
Recipients' medications	
Beta-blocker, *n* (%)	12 (75)
ACEI or ARB, *n* (%)	11 (69)
Statin, *n* (%)	12 (75)
Cyclosporine, *n* (%)	6 (38)
Tacrolimus, *n* (%)	10 (63)
Recipients' laboratory parameters	
White blood cells, × $10^3/\mu L$	13.4 ± 3.2
Hemoglobin, g/dL	11.4 ± 1.5
Platelets, × $10^3/\mu L$	24.4 ± 11.3
Serum sodium, mEq/L	134 ± 4
Serum potassium, mEq/L	4.7 ± 0.4
Serum BUN, mg/dL	31 ± 21
Serum creatinine, mg/dL	1.1 ± 0.7
Serum albumin, g/dL	3.1 ± 0.3
Serum total bilirubin, mg/dL	1.4 ± 1.1
Serum CRP, mg/dL	2.4 ± 1.2
Plasma BNP, pg/mL	358 ± 319
Recipients' echocardiographic parameters	
LVDd, mm	41 ± 6
LVDs, mm	26 ± 4
LVEF, %	67 ± 6
AR, grade	0.1 ± 0.3
MR, grade	0.1 ± 0.3
TR, grade	0.3 ± 0.5
E/e'	12.5 ± 3.5
Recipients' hemodynamic parameters	
mRAP, mmHg	7 ± 4
mPAP, mmHg	19 ± 6
PCWP, mmHg	13 ± 4
CI, L/min/m^2	2.8 ± 0.4

HF indicates high frequency; HTx, heart transplantation; PF, pulsatile flow; CF, continuous flow; LVAD, left ventricular assist device; NGSP, national glycohemoglobin standardization program; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BUN, blood urea nitrogen; CRP, C-reactive protein; BNP, B-type natriuretic peptide; LVDd, left ventricular diastolic diameter; LVDs, left ventricular systolic diameter; LVEF, left ventricular ejection fraction; AR, aortic regurgitation; MR, mitral regurgitation; TR, tricuspid regurgitation; E/e', ratio of early diastolic transmitral flow velocity to mitral annular velocity at the lateral wall; mRAP, mean right atrial pressure; mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; and CI, cardiac index.

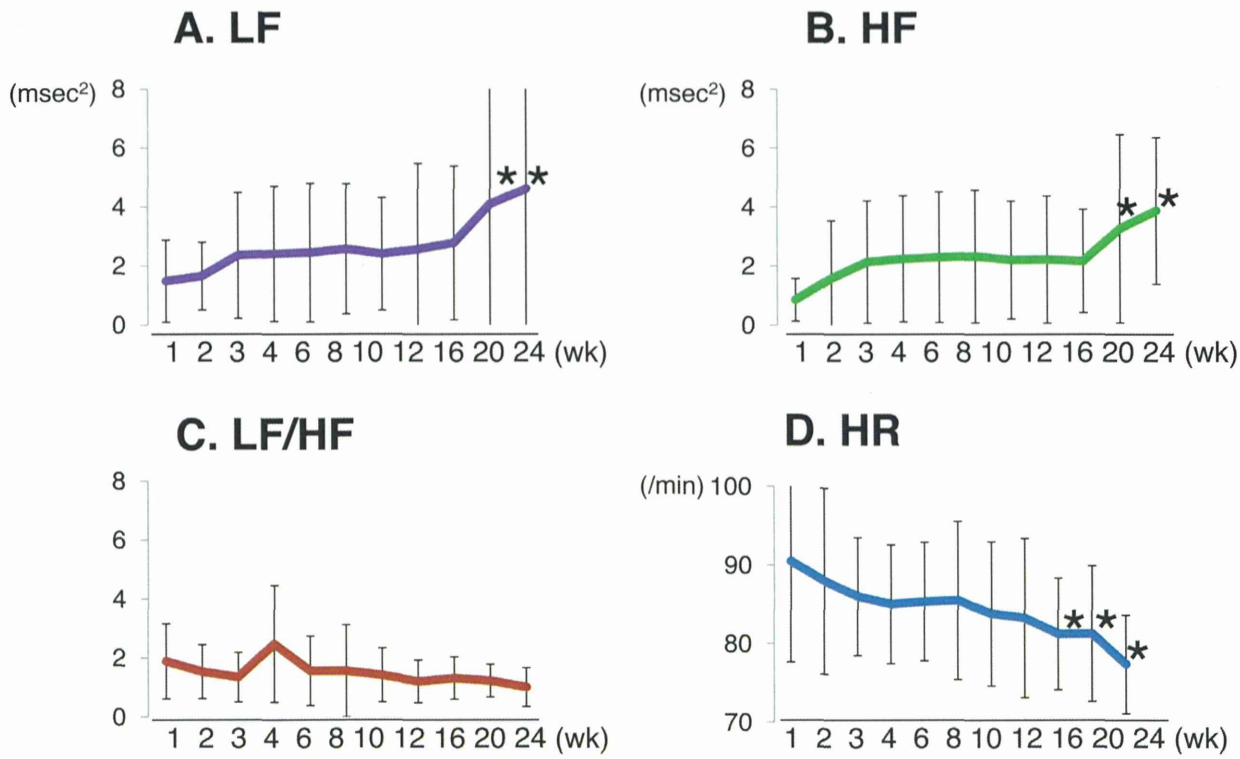

Figure. Time courses of HRV parameters from 1 week to 24 weeks after HTx, ie, LF (**A**), HF (**B**), LF/HF (**C**), HR (**D**). *$P < 0.05$ by Dunnett's test compared with those of 1 week post-HTx when repeated analysis of variance proved significance.

patients (63%). Left ventricle contractility was well preserved without any significant valvular diseases, and hemodynamics were stable.

Time courses of HRV parameters during the study period (Early-stage : 1-24 weeks): LF and HF increased gradually and reached significantly higher levels after postoperative 20 weeks (Figure A and B). LF/HF, which represents sympathetic activity, remained unchanged during the study period (Figure C). HR was 91 ± 10 beats per minute (bpm) at baseline and decreased gradually down to 80 ± 10 bpm (Figure D).

Relationship between achieved HF and baseline parameters (Table II): Among baseline parameters including donor, transplant surgery, and recipient data, only a shorter cardiopulmonary bypass time was significantly associated with higher HF levels at 6 months after HTx ($P = 0.035$, $r = -0.530$).

Relationship between achieved HF and clinical parameters at 6 months after HTx: Clinical parameters at 6 months after HTx are shown in Table III. HF levels correlated with HR levels and %changes in HR levels among post-HTx recipient clinical parameters at 6 months including laboratory, echocardiographic, and hemodynamic parameters (Table IV).

Discussion

We demonstrated here that parasympathetic reinnervation gradually occurs < 6 months after HTx by HRV analyses. Shorter cardiopulmonary bypass time correlated with improvement of parasympathetic reinnervation among the recipient baseline parameters, and improved parasympathetic reinnervation was associated with recovery of tachycardia at 6 months after HTx.

Time courses of reinnervation: Earlier studies demonstrated that sympathetic reinnervation occurs approximately > 1 year after HTx mainly using positron emission tomography imaging or measurement of transcardiac norepinephrine release induced by intravenous tyramine.[3,7,8,11,18] However, these modalities cannot analyze parasympathetic nerve activity.

Power spectral analyses of HRV is a noninvasive method to assess autonomic cardiac modulation and provides information on not only sympathetic but also parasympathetic activity of the sinus node.[19] A few investigators demonstrated parasympathetic reinnervation at several years after HTx using cross-sectional or paired-time data.[10,20,21] Our successive analyses of HRV data demonstrated for the first time that parasympathetic reinnervation occurred earlier than 1 year after HTx. Our result that no significant sympathetic reinnervation occurred < 6 months after HTx was consistent with the findings of the previous studies discussed above. Six months may be too short for sympathetic reinnervation.

Factors that influence reinnervation: Autonomic reinnervation does not occur in the same manner in each recipient.[10,11] What affects autonomic reinnervation after HTx? Bengel, *et al* showed that the regenerational capacity of the cardiac sympathetic nervous system was reduced if the recipient had diabetes mellitus, because impaired glucose handling adversely affected autonomic nerve activity and regeneration.[22] They also demonstrated in another paper that sympathetic reinnervation was more likely with a younger donor/recipient and a fast and uncomplicated HTx procedure.[12] Neuronal regeneration is de-

Table II. Correlation Between Baseline Parameters and Parasympathetic Activity at 6 Months After HTx

Variables	*P*	*r*
Donor parameters		
Age, years	0.898	0.035
Male, *n* (%)	0.585	-0.148
Transplant surgery		
Duration of allograft ischemia, minutes	0.371	-0.240
Cardiopulmonary bypass time, minutes	0.035*	-0.530
Aortic cross-clamp time, minutes	0.639	-0.127
Recipients' pre-HTx parameters		
PF LVAD, *n* (%)	0.320	0.266
CF LVAD, *n* (%)	0.320	0.266
Duration of VAD treatment, days	0.971	-0.010
Etiology of ischemia, *n* (%)	0.787	-0.073
Recipients' demographic parameters		
Age, years	0.293	0.281
Male, *n* (%)	0.408	0.222
Body mass index	0.730	-0.094
Systolic blood pressure, mmHg	0.534	0.123
Diastolic blood pressure, mmHg	0.644	0.154
HbA_{1c} (N), %	0.248	-0.307
Recipients' medications		
Beta-blocker, *n* (%)	0.078	-0.453
ACEI or ARB, *n* (%)	0.154	-0.374
Statin, *n* (%)	0.597	0.143
Cyclosporine, *n* (%)	0.456	-0.201
Tacrolimus, *n* (%)	0.456	0.201
Recipients' laboratory parameters		
White blood cells, $\times 10^3/\mu L$	0.432	0.211
Hemoglobin, g/dL	0.156	0.372
Platelets, $\times 10^3/\mu L$	0.645	-0.125
Serum sodium, mEq/L	0.380	0.236
Serum potassium, mEq/L	0.425	-0.215
Serum BUN, mg/dL	0.712	0.100
Serum creatinine, mg/dL	0.763	0.082
Serum albumin, g/dL	0.372	-0.239
Serum total bilirubin, mg/dL	0.886	0.039
Serum CRP, mg/dL	0.723	0.121
Plasma BNP, pg/mL	0.861	0.048
Recipients' echocardiographic parameters		
LVDd, mm	0.397	0.227
LVDs, mm	0.858	0.049
LVEF, %	0.231	0.317
AR, grade	0.214	0.143
MR, grade	0.793	-0.071
TR, grade	0.541	-0.165
E/e'	0.762	-0.082
Recipients' hemodynamic parameters		
mRAP, mmHg	0.827	-0.059
mPAP, mmHg	0.743	-0.089
PCWP, mmHg	0.935	0.022
CI, L/min/m^2	0.313	-0.269

Abbreviations as in Table I. *$P < 0.05$ by Pearson's product-moment correlation coefficients.

Table III. Clinical Parameters at 6 Months After HTx

Variables	
Recipients' laboratory parameters	
White blood cells, $\times 10^3/\mu L$	6.0 ± 1.8
Hemoglobin, g/dL	10.6 ± 1.5
Platelets, $\times 10^3/\mu L$	25.2 ± 9.1
Serum sodium, mEq/L	137 ± 4
Serum potassium, mEq/L	4.7 ± 0.6
Serum BUN, mg/dL	19 ± 7
Serum creatinine, mg/dL	1.2 ± 0.5
Serum albumin, g/dL	4.0 ± 0.5
Serum total bilirubin, mg/dL	0.5 ± 0.2
Serum CRP, mg/dL	0.3 ± 0.3
Plasma BNP, pg/mL	139 ± 126
Recipients' electrocardiographic parameters	
PQ time, msec	148 ± 17
QRS time, msec	99 ± 19
Heart rate, bpm	80 ± 9
%changes in heart rate, %	-11 ± 10
Recipients' echocardiographic parameters	
LVDd, mm	42 ± 5
LVDs, mm	26 ± 5
LVEF, %	69 ± 8
AR, grade	0.3 ± 0.4
MR, grade	0.3 ± 0.5
TR, grade	0.3 ± 0.5
E/e'	11.4 ± 3.2
Recipients' hemodynamic parameters	
mRAP, mmHg	4 ± 2
mPAP, mmHg	16 ± 4
PCWP, mmHg	9 ± 3
CI, L/min/m^2	3.4 ± 0.5

Abbreviations as in Table I.

pendent on neurotrophins, which are neuronal growth factors produced and released by target tissue.[23] Aging, extensive surgical dissection, and prolonged tissue ischemia may reduce the availability of target-derived neurotrophic factors.[24]

We demonstrated for the first time that a shorter cardiopulmonary time was correlated with more improved parasympathetic reinnervation. A longer cardiopulmonary time indicates complexity of the operation, more injured tissue due to extensive adhesiolysis, or a longer warm ischemic time for the donor heart, which may adversely affect parasympathetic reinnervation in the same manner as that of regeneration of the sympathetic system. Neither diabetes mellitus nor age was associated with parasympathetic reinnervation, most likely because the recipients were all under 60 years-old, and were not complicated with severe diabetes mellitus.

Clinical courses accompanied by reinnervation: Several authors reported a correlation between sympathetic reinnervation and improvement of exercise tolerability demonstrated by improved peak oxygen consumption, recovery time or peak levels of HR, or exercise duration during a cardiopulmonary exercise test.[2,3,8] In contrast, Bengel, *et al* reported that there were no significant differences in hemodynamics between denervated and reinnervated allografts under resting conditions.[12]

No report has discussed the functional outcomes of parasympathetic reinnervation in HTx recipients. We demonstrated that parasympathetic reinnervation was associated with decreased HR at 6 months after HTx. Whether decreased HR by parasympathetic reinnervation improves the prognosis or quality of life of the recipient would be a future concern.

Study limitations: 1. The present study was performed at a single center in a retrospective manner in a small number of recipients under short-term observation. Longer observation of a larger number of recipients would be a future concern, but it may be difficult considering the shortage of donor hearts in Japan. 2. The association between parasympathetic reinnervation and functional outcome under exercise testing or prognostic efficacy should be investigated in the future.

In conclusion, parasympathetic reinnervation occurs along with recovery of tachycardia < 6 months after HTx, es-

Table IV. Relationship Between Clinical Parameters and Parasympathetic Activity at 6 Months After HTx

Variables	*P*	*r*
Recipients' laboratory parameters		
White blood cells, $\times 10^3/\mu L$	0.600	-0.142
Hemoglobin, g/dL	0.088	0.440
Platelets, $\times 10^3/\mu L$	0.269	-0.294
Serum sodium, mEq/L	0.802	0.068
Serum potassium, mEq/L	0.448	-0.204
Serum BUN, mg/dL	0.340	-0.255
Serum creatinine, mg/dL	0.874	0.043
Serum albumin, g/dL	0.722	0.097
Serum total bilirubin, mg/dL	0.330	0.261
Serum CRP, mg/dL	0.512	0.143
Plasma BNP, pg/mL	0.313	0.269
Recipients' electrocardiographic parameters		
PQ time, msec	0.441	-0.208
QRS time, msec	0.859	-0.048
Heart rate, bpm	0.035*	-0.514
%changes in heart rate, %	0.007*	-0.644
Recipients' echocardiographic parameters		
LVDd, mm	0.715	0.099
LVDs, mm	0.848	0.052
LVEF, %	0.991	0.003
AR, grade	0.367	-0.242
MR, grade	0.990	-0.003
TR, grade	0.569	0.154
E/e'	0.589	-0.147
Recipients' hemodynamic parameters		
mRAP, mmHg	0.684	-0.110
mPAP, mmHg	0.090	-0.437
PCWP, mmHg	0.288	-0.283
CI, L/min/m^2	0.563	-0.156

Abbreviations as in Table I. *$P < 0.05$ by Pearson's product-moment correlation coefficient.

pecially in recipients with a shorter cardiopulmonary bypass time. The clinical benefit of improved parasympathetic reinnervation should be investigated in the future.

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ORIGINAL ARTICLE
Heart Failure

Status 2 Patients Had Poor Prognosis Without Mechanical Circulatory Support

– Indications for Device Implantation –

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Background: Indication for mechanical circulatory support (MCS) has been a matter of debate in less sick status 2 patients.

Methods and Results: Data were obtained from 183 consecutive patients assigned to stage D heart failure (HF) who were evaluated by the institutional review board of the University of Tokyo Hospital and then listed for heart transplantation as status 1 or 2 of the Japan Organ Transplant Network. Patients with status 2 (n=38) had a prognosis as poor as those dependent on inotropes (n=54) or MCS (n=91; P=0.615, log-rank test), and only 4 of them had eventual ventricular assist device (VAD) implantation (10.5%). Patients who eventually received VAD (n=92) had better 4-year survival than those without MCS among status 1 and 2 (P=0.030, log-rank test). On Cox regression analysis plasma B-type natriuretic peptide (BNP) >740 pg/ml was the only significant predictor for 4-year survival among the status 2 group (P=0.014; hazard ratio, 8.267). Ten patients with status 2 died: 6 due to acute hemodynamic compromise and 4 due to ventricular fibrillation.

Conclusions: Prognosis in status 2 patients was as poor as that of those dependent on inotrope infusion or VAD, mostly because of out-of-hospital sudden death without MCS. Status 2 patients with considerably high plasma BNP may be good candidates for continuous flow VAD therapy. (*Circ J* 2014; **78:** 1396–1404)

Key Words: Heart failure; Heart transplantation; INTERMACS

Survival in patients with stage D heart failure (HF) has remained unsatisfactory in the era of guideline-directed optimal medical therapy consisting of β-blockers, angiotensin-converting enzyme inhibitors (ACEI), aldosterone antagonists, and cardiac resynchronization therapy with or without defibrillators (CRT-D).[1] Although heart transplantation (HTx) is the ultimate solution for such refractory patients, approximately 90% of Japanese recipients eventually require implantation of ventricular assist device (VAD) for bridge to HTx (BTT) because of the long waiting period due to severe donor shortage.[2]

The current Japanese reimbursement system requires the approval of the institutional review board for the eligibility of HTx and successive HTx listings on the Japan Organ Transplant (JOT) Network prior to continuous flow (CF) VAD implantation.[3] In Japan, extracorporeal (EC) VAD had been widely used as the only durable device until CF LVAD became available in 2011, and because of its EC nature, EC VAD was usually implanted under unstable hemodynamics. Nowadays EC VAD is still implanted in patients with cardiogenic shock as bridge to decision, and such patients may be listed for HTx after confirming eligibility later.[4] Currently, EC VAD is also indicated for patients with small body surface area. HTx recipients listed on JOT Network are classified into 2 groups according to patient condition, that is, (1) 'status 1' for patients dependent on mechanical support including VAD or i.v. infusion of inotropes, equivalent to INTERMACS profile 1–3 or the United Network for Organ Sharing (UNOS)

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Table 1. Patient Characteristics

	Total (n=183)	Status 2 (n=38)	Status 1 VAD (−) (n=54)	P-value vs. Status 2	Status 1 VAD (+) (n=91)	P-value vs. Status 2
Demographic parameters						
Age (years)	40.0±14.4	38.8±17.7	40.7±14.1	0.728	40.1±13.3	0.895
Male	135 (74.2)	26 (68.4)	46 (85.2)	0.075	63 (69.2)	0.075
Body surface area (m^2)	1.56±0.27	1.53±0.38	1.58±0.25	0.132	1.57±0.22	0.098
Etiology of ischemia	27 (14.8)	4 (10.5)	5 (9.3)	0.412	18 (19.8)	0.024†
SBP (mmHg)	90.5±11.1	87.6±9.7	86.0±8.2	0.760	94.4±11.8	0.003*
Heart rate (beats/min)	83.4±14.8	82.7±14.5	84.2±17.1	0.885	83.1±13.6	0.990
History of NSVT	65 (35.7)	13 (34.2)	27 (50.0)	0.026†	25 (27.4)	0.423
Etiology						
DCM	114 (62.3)	22 (57.9)	39 (72.2)	–	53 (58.2)	–
ICM	29 (15.8)	4 (10.6)	5 (9.3)	–	20 (22.0)	–
ACHD	3 (1.6)	2 (5.3)	0 (0)	–	1 (1.1)	–
dHCM	14 (7.7)	4 (10.6)	6 (11.4)	–	4 (4.4)	–
Secondary cardiomyopathy	5 (2.7)	2 (5.3)	1 (1.9)	–	2 (2.2)	–
Cardiac sarcoidosis	3 (1.6)	2 (5.3)	1 (1.9)	–	0 (0)	–
RCM	3 (1.6)	2 (5.3)	0 (0)	–	1 (1.1)	–
Myocarditis	12 (6.6)	0 (0)	2 (3.8)	–	10 (11.0)	–
Concomitant treatment						
Furosemide (mg daily)	39.1±33.7	47.4±32.0	59.6±36.7	0.128	23.3±23.3	<0.001*
Furosemide	150 (82.0)	37 (97.4)	52 (96.3)	0.945	61 (67.0)	<0.001†
Aldosterone antagonist (mg daily)	33.3±21.7	37.2±23.3	31.3±20.9	0.405	32.9±21.6	0.571
Aldosterone antagonist	157 (85.8)	36 (94.7)	46 (85.2)	0.734	75 (82.4)	0.834
β-blocker (mg daily)	11.9±11.8	10.7±7.1	7.4±5.8	0.354	15.1±14.8	0.111
β-blocker	172 (94.0)	36 (94.7)	51 (94.4)	0.998	85 (93.4)	0.984
ACEI/ARB (mg daily)	3.0±2.8	3.5±3.1	3.4±3.0	0.986	2.6±2.6	0.231
ACEI/ARB	153 (83.6)	35 (92.1)	50 (92.6)	0.945	68 (74.7)	0.142
CRT-D	77 (42.3)	22 (57.9)	34 (63.0)	0.634	21 (23.1)	<0.001†
Laboratory parameters						
White blood cells ($\times10^3/\mu l$)	6.3±1.7	5.9±1.5	6.3±1.5	0.564	6.4±1.9	0.280
Hemoglobin (g/dl)	11.8±2.0	12.9±1.9	12.3±2.1	0.281	11.0±1.6	<0.001*
Platelets ($\times10^3/\mu l$)	22.4±9.1	21.5±7.1	22.0±8.7	0.954	23.1±10.0	0.641
Serum sodium (mEq/L)	136.2±4.5	136.0±5.3	134.4±4.1	0.187	137.4±4.0	0.209
Serum potassium (mEq/L)	4.2±0.4	4.2±0.4	4.3±0.4	0.837	4.2±0.3	1.000
Serum BUN (mg/dl)	23.1±4.3	23.1±4.3	25.1±5.8	0.096	22.2±4.0	0.001*
Serum creatinine (mg/dl)	0.9±0.4	0.9±0.4	1.0±0.4	0.275	0.8±0.3	0.060
Serum albumin (g/dl)	3.8±0.5	4.0±0.6	3.8±0.5	0.150	3.6±0.5	<0.001*
Serum GOT (IU/L)	28.7±12.6	29.1±12.3	27.1±12.3	0.727	29.4±12.9	0.990
Serum GPT (IU/L)	26.3±17.2	27.3±18.1	27.1±18.1	0.964	24.8±15.1	0.746
Serum LDH (IU/L)	335.8±186.3	260.9±165.1	252.0±83.2	0.966	417.6±204.2	<0.001*
Serum total bilirubin (mg/dl)	1.1±0.8	1.1±0.6	1.3±0.7	0.464	1.1±0.9	0.985
Plasma BNP (log_{10}pg/ml)	2.83±3.03	2.90±2.81	3.04±3.20	0.353	2.58±2.77	0.099
Echocardiographic parameters						
LV diastolic diameter (mm)	62.2±17.0	65.3±15.5	71.4±15.5	0.164	55.5±16.7	0.003*
LV systolic diameter (mm)	55.3±16.8	57.6±15.9	64.5±16.7	0.087	48.8±16.1	0.011*
IVSD (mm)	7.8±1.7	7.6±1.7	7.8±1.9	0.933	7.9±1.5	0.610
PWD (mm)	8.0±1.7	7.9±1.7	7.9±1.9	0.989	8.2±1.5	0.684
LVMI (g/m^2)	150.5±77.8	171.6±68.6	177.2±71.0	0.932	126.7±76.1	<0.001*
Ejection fraction (%)	23.7±11.0	24.7±13.9	21.1±8.7	0.058	25.0±10.4	0.970
AR (grade)	0.2±0.4	0.2±0.6	0.2±0.4	0.977	0.2±0.4	0.998
MR (grade)	1.2±0.9	1.3±0.7	1.6±0.9	0.257	0.8±1.0	0.003*
TR (grade)	1.1±0.7	1.1±0.6	1.3±0.7	0.491	1.0±0.7	0.678
Hemodynamic parameters						
mRAP (mmHg)	8.1±4.4	6.9±8.6	6.3±4.3	0.321	8.4±4.6	0.218
mPAP (mmHg)	23.8±8.9	24.9±8.6	28.0±10.5	0.068	21.7±6.3	0.108
PCWP (mmHg)	16.8±8.1	18.4±7.9	21.9±8.6	0.070	13.2±5.8	0.001*
CI ($L\cdot min^{-1}\cdot m^{-2}$)	2.3±0.7	2.2±0.5	2.0±0.4	0.427	2.5±0.7	0.025*
PVR (WU)	2.2±1.2	2.1±1.4	2.5±1.1	0.169	2.1±1.1	0.987
RVSWI (g/m^2)	6.2±3.2	6.4±2.6	7.3±3.6	0.374	5.4±3.0	0.250
CVP/PCWP	0.5±0.3	0.4±0.2	0.4±0.2	0.971	0.7±0.4	<0.001*

(Table 1's footnote is on the next page.)