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Suppressive effects of conversion from mycophenolate mofetil to everolimus for the development of cardiac allograft vasculopathy in maintenance of heart transplant recipients☆,☆☆

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ABSTRACT

Background: Whether converting to everolimus (EVL) from mycophenolate mofetil (MMF) during the maintenance period after heart transplantation (HTx) reduces cardiac allograft vasculopathy (CAV) progression remains unclear. We sought to determine the effect of converting from MMF with standard-dose calcineurin inhibitors (CNIs) to EVL with low-dose CNIs on CAV progression.

Methods: We retrospectively reviewed the medical records of 63 HTx recipients who survived at least at 1 year after HTx. Twenty-four recipients were converted from MMF to EVL (EVL group, 2.2 ± 2.3 years after HTx), while 39 recipients were maintained on MMF (MMF group, 2.4 ± 2.2 years after HTx). The EVL group underwent three-dimensional intravascular ultrasound (3D-IVUS) analysis before and 1 year after conversion to EVL, and these data were compared with data from 2 consecutive IVUS in the MMF group.

Results: IVUS indices in the EVL group at 1 year after conversion did not show increased CAV development, whereas a significant increase in %plaque volume ($p = 0.006$) and decrease in lumen volume ($p < 0.001$) were observed in the MMF group. EVL conversion was significantly associated with smaller increases in %plaque volume ($p = 0.004$) and smaller decreases in lumen volume ($p = 0.017$). IVUS indices in the late EVL conversion group (≥2 years) also did not exhibit increased CAV development, while those in the MMF group did.

Conclusions: Conversion to EVL from MMF in maintenance periods after HTx may decrease the rate of CAV progression based on IVUS indices.

Abbreviations: %PVI, percent plaque volume index; 3D-IVUS, three-dimensional intravascular ultrasound; AZA, azathioprine; CAV, cardiac allograft vasculopathy; CMV, cytomegalovirus; CNIs, calcineurin inhibitors; EEM, external elastic membrane; EVL, everolimus; GFR, glomerular filtration rate; HTx, heart transplantation; ISHLT, International Society of Heart and Lung Transplantation; LVI, lumen volume index; MIT, maximal intimal thickness; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; NCVC, National Cerebral and Cardiovascular Center; PVI, plaque volume index; VVI, vessel volume index.

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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1. Introduction

Cardiac allograft vasculopathy (CAV) is the leading cause of late morbidity and mortality in heart transplant recipients, accounting for one-third of all-cause mortality at 5 years [1]. The pathophysiology of CAV is closely linked to both immunologic factors, such as alloreactive T-cell and antibody activation, and non-immunologic factors, including pre-transplant coronary artery disease, cytomegalovirus (CMV) infection, older age of the donor and recipient, recipient's conventional risk factor (e.g. hyperlipidemia, hypertension and diabetes mellitus) and ischemia/reperfusion injury [2]. This complex etiology makes it difficult to prevent and suppress the development of CAV.

Immunosuppression after heart transplantation (HTx) has traditionally consisted of calcineurin inhibitors (CNIs) combined with mycophenolate mofetil (MMF) or azathioprine (AZA) and corticosteroids [1]. Mammalian target of rapamycin (mTOR) inhibitors, the novel immunosuppressants such as everolimus (EVL) and sirolimus, are expected to suppress CAV progression [3,4]. Previous studies have examined the influence of mTOR inhibitors on CAV by comparing them with various baseline immunosuppressive drugs, such as AZA [4], AZA and MMF [3–5], CNI-free [6,7], and add-on regimens [8]. However, concomitant immunosuppression with EVL may affect the suppressive effects of EVL on CAV progression [8]. Recently, MMF has replaced AZA due to inferior outcomes [9], and so a direct comparison between MMF and EVL is required [10,11]. Recent clinical trials on de novo heart transplant recipients have indicated that EVL with reduced-dose CNIs is more effective than MMF with standard-dose CNIs for suppressing first-year CAV progression after HTx [10,11]. However, the effects of converting to EVL from MMF in maintenance periods after HTx are still controversial [3,5–8]; furthermore, the underlying mechanism for the suppressive effect of EVL on CAV progression remains unclear [10].

The aim of the present study was to elucidate the effect of converting from MMF with standard-dose CNIs to EVL with low-dose CNIs on CAV progression using three-dimensional intravascular ultrasound (3D-IVUS).

2. Methods

2.1. Patient management and selection

We retrospectively reviewed the medical records of all post-HTx recipients at the National Cerebral and Cardiovascular Center (NCVC) in Japan between July 1993 and March 2013.

All de novo heart transplant recipients in our institution received triple immunosuppressive therapy consisting of CNIs (i.e., cyclosporine or tacrolimus), MMF, and corticosteroids [12]. We regulated immunosuppressive drug dosage based on blood trough concentrations. Standard target trough levels were as follows: cyclosporine, 350–450 ng/mL for the first month, 250–350 ng/mL between 1 and 3 months, 200–300 ng/mL between 3 and 12 months, and 100–250 ng/mL after the 1-year follow-up; tacrolimus, 9–12 ng/mL for the first 3 months, 8–9 ng/mL between 3 and 6 months, and 6–8 ng/mL after the 6-month follow-up. Tacrolimus was used as an alternative to cyclosporine as the primary immunosuppressant beginning in 2005.

Since 2007, we have primarily considered converting from MMF with standard-dose CNIs to EVL with low-dose CNIs for the following recipients: 1) recipients with impaired renal function (glomerular filtration rate [GFR] <60 mL/min/1.73 m^2); 2) those with increases in or an initially large maximal intimal thickness (MIT) on routine IVUS examinations; and 3) those with MMF-related leukopenia. According to our protocol for EVL conversion with low-dose CNIs, the standard target trough levels were as follows: EVL, 6–8 ng/mL; reduced-dose cyclosporine, 50% of standard blood concentrations; and reduced-dose tacrolimus, 3–4 ng/mL. The conversion to EVL from MMF was initiated with EVL 1.0–1.5 mg/day while MMF was withdrawn. The trough levels of EVL were evaluated at 1 week after initiation. Once target trough levels of EVL (6–8 ng/mL) were achieved, the tacrolimus or cyclosporine dose was reduced to obtain target trough levels [13].

Routine endomyocardial biopsies were performed weekly for 3 weeks after HTx, every 2 weeks from 3 weeks to 2 months, at 3 months, every 1.5 months from 3 months to 6 months, every 3 months from 6 months to 12 months, and then at 6-month intervals until the end of the fifth year, after which we performed endomyocardial biopsy every year. An International Society of Heart and Lung Transplantation (ISHLT) grade of 2R or greater acute cellular rejection on routine endomyocardial biopsy was treated with augmented immunosuppression and intravenous steroids [12,14,15]. Follow-up endomyocardial biopsies were performed at 14 to 21 days in treated cases.

Coronary angiography and IVUS examinations were performed 5–12 weeks after HTx and repeated to evaluate CAV every year. Coronary angiography was used to classify the severity of CAV as ISHLT CAV 0 (not significant), CAV 1 (mild), CAV 2 (moderate), or CAV 3 (severe) on the basis of the ISHLT guidelines [16]. A 40-MHz mechanical ultrasound transducer (View it®, Terumo, Tokyo, Japan) was advanced into a distal portion of the left anterior descending artery. Continuous ultrasound imaging was acquired at a constant rate of 1.0 mm/s to evaluate the coronary artery. Images were digitized for analysis by a researcher (T.W.) who was blinded to the clinical characteristics and treatment status of the patients. IVUS images were stored on S-VHS tapes for offline 3D IVUS analysis (Nicoras T2000® Ver. 2.1, Terumo, Tokyo, Japan). Using cross-sectional IVUS images, we compared changes in the MIT, which is known to impact long-term outcome after HTx [17–19], and coronary vessel, plaque, and lumen volumes based on 3D-IVUS images in both the EVL and MMF groups.

Seventy-four recipients who survived more than 1 year post-HTx were initially screened for inclusion (Fig. 1). Of these, 11 recipients were excluded because they lacked data from two consecutive IVUS studies. Of the remaining 63 recipients, 24 were converted from MMF with standard-dose CNIs to EVL with reduced-dose CNIs (EVL group). Of these, 17 recipients (70.8%) were converted to EVL because of CAV development, and five (20.8%) and two recipients (8.3%) were converted to EVL because of CNI-induced nephropathy and MMF-related leukopenia, respectively. The other 39 recipients remained on MMF with standard-dose CNIs (MMF group). Baseline characteristics of the recipients included in this study were collected at "study entry," which was defined as the time of the earlier of the two most recent consecutive IVUS examinations in the MMF group and as the time of the last IVUS examination before conversion in the EVL group. We analyzed data from the two most recent consecutive IVUS examinations to include all recipients who continued taking MMF as the control group. In the EVL group, IVUS data before EVL conversion (study entry) and at the 1-year follow-up after conversion were analyzed. Therefore, changes in IVUS parameters before and after conversion in the EVL group were compared with changes in the parameters of the two most recent consecutive IVUS examinations in the MMF group. There were no significant differences in the length of time between HTx and study entry between the two groups (mean, 2.2 years after HTx for the EVL group [range, 0.1–9.0 years] and mean, 2.4 years for the MMF group [range, 0.1–7.9 years], $p = 0.614$). To account for the timing of EVL conversion, study subjects were sub-classified into two groups according to the length of time between HTx and study entry. The "early" cohort included subjects enrolled within 2 years post-HTx, and the "late" cohort included the other subjects, who were enrolled more than 2 years post-HTx.

Because 14 recipients (7 in each group) underwent HTx in other countries, detailed information on their donors (i.e., donor age, sex, status of CMV infection mismatch, and cold ischemia time) were unavailable. In Japan, the Organ Transplant Law was enacted in October 1997 [12,20], and the first HTx in Japan was performed in February 1999 from a brain-dead donor in accordance with this law. From then until March 2013, 185 HTxs were performed in Japan, including 54 cases at our institute. In the present study, we included 49 of those 54 patients who underwent HTx at our institute and 14 recipients who underwent HTx in the United States and Germany on the basis of official procedures between 1993 and 2009. The ethics committee of the NCVC of Japan approved this study. Informed consent was obtained from all participants (IRB number M25-020 at NCVC).

2.2. Intravascular ultrasound measurements (Fig. 2)

We compared changes in IVUS data obtained at study entry and at the 1-year follow-up between both groups. Cross-sectional images of the left anterior descending artery spaced precisely 1 mm apart were selected for measurement. The maximum length measured was 50 mm of the left anterior descending artery, from the distal portion to the ostium. MIT was measured at the site with the greatest intimal thickness in the observed length. Plaque area was defined as the difference between the area occupied by the lumen and external elastic membrane (EEM) borders. Volumetric analyses were calculated as the summation of each area (vessel, plaque, and lumen). Each volume was standardized to account for differences in segment length between different subjects (vessel, plaque, and lumen volume indexes; VVI, PVI and LVI, respectively), and was calculated as: volumetric value/measured length (mm^3/mm). Percent plaque volume index (%PVI) was calculated as: (PV/VV) × 100%. Change in percent plaque volume was calculated as: (percent plaque volume at follow-up) − (percent plaque volume at study entry). In order to adjust for differences in the initial IVUS data between the EVL and MMF groups, relative changes for each volumetric data measure were calculated as: [(volumetric index at follow-up − volumetric index at entry) / (volumetric index at entry)] × 100%.

2.3. Statin therapy

Statin therapy was generally initiated within 2 months after HTx for all post-transplant recipients, regardless of cholesterol level, except for recipients who experienced adverse effects due to statin therapy. Pravastatin was generally used [21], but if the lipid profile worsened or if CAV progression was observed with conventional statin use, the statin dosage was increased or pravastatin was exchanged for a more powerful agent (i.e., a "strong statin"). We used atorvastatin, rosuvastatin, and pitavastatin as strong statins. Doses of at least 20 mg/day for pravastatin, 20 mg/day for atorvastatin, 5 mg/day for rosuvastatin, and 4 mg/day for pitavastatin were classified as "high-dose" statins. "Intensive statin therapy" was defined as follows: (1) initiating statin therapy during the study period in recipients who did not take statins at study entry, (2) increasing the statin dosage during the study period, and (3) converting to a strong statin from pravastatin during the study period.

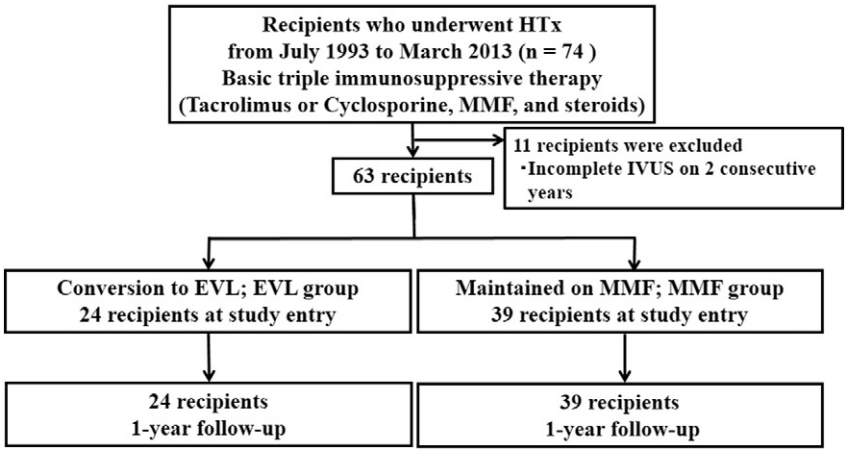

Fig. 1. Study flow-chart. EVL = everolimus; HTx = heart transplantation; IVUS = intravascular ultrasound; MMF = mycophenolate mofetil.

2.4. Statistical analysis

Data are expressed as means ± standard deviations for normally distributed continuous variables, medians with interquartile ranges for non-normally distributed continuous variables, and numbers (percentages) for categorical variables. The chi-squared test was used to test for differences in categorical variables between groups. Comparisons between study entry and follow-up IVUS data were determined with paired t-tests or Wilcoxon signed rank tests within each treatment group. Changes in volumetric indices between study entry and the 1-year follow-up were treated as categorical variables by defining the median value as the cut-off point for relative changes in each volumetric IVUS parameter (−4.3%, 4.8%, 0.7% and −3.9%, for the changes in vessel, plaque, percent plaque, and lumen volume index, respectively). Regarding changes in MIT, a cut-off value of a 0.3-mm increase was chosen. The associations between change in IVUS parameters and EVL conversion were investigated using a logistic regression analysis. A multivariable logistic regression analysis was conducted using the recipients' age, sex, strong statin use at the 1-year follow-up, intensive statin therapy, and time since HTx for Model 1 and the variables with p-values < 0.05 in comparison between the two groups as shown in Table 1 for Model 2. A p-value < 0.05 was considered significant. All analyses were performed using commercial software (STATA® version 13, Stata Corporation, College Station TX, USA).

3. Results

3.1. Baseline clinical demographic

Table 1 shows baseline demographics, medications, and laboratory data for both groups. The prevalence of previous hypertension (p = 0.038) and antiplatelet therapy (p = 0.044) at study entry were significantly higher in the MMF group. Serum hemoglobin A1c level was significantly higher in the EVL group (p = 0.019). Renal function, which was assessed by creatinine value and estimated GFR (eGFR) [22], was comparable between the two groups.

Regarding statin therapy (Table 2), there were no significant differences between groups with respect to statin dose and type at study entry. However, the EVL group had a higher rate of strong statins replacing pravastatin at the 1-year follow-up (p = 0.005), as four recipients in the EVL group and two in the MMF group had been converted from pravastatin to a strong statin by the 1-year follow-up. Additionally, the EVL group had a higher incidence of intensive statin therapy at the 1-year follow-up (p = 0.021).

3.2. Severity of CAV at study entry

The severity of CAV on coronary angiography at study entry was comparable between the two groups (p = 0.256); 16 (66.7%) recipients were graded as CAV 0 and 8 (33.3%) as CAV 1 in the EVL group, and 31 (79.4%) recipients were graded as CAV 0 and 8 (20.5%) as CAV 1 in the MMF group. On the other hand, the prevalence of CAV determined by IVUS (defined as MIT > 0.5 mm) was higher in the EVL group than in the MMF group (91.7% vs. 56.4%, p = 0.003).

3.3. IVUS measurements in the EVL and MMF groups

Table 3 summarizes the IVUS measurements at study entry and at the 1-year follow-up for the two groups. CAV on IVUS was more developed in the EVL group than in the MMF group at study entry, as indicated by significantly greater PVI, %PVI, and MIT (all p < 0.001). LVI was 20%

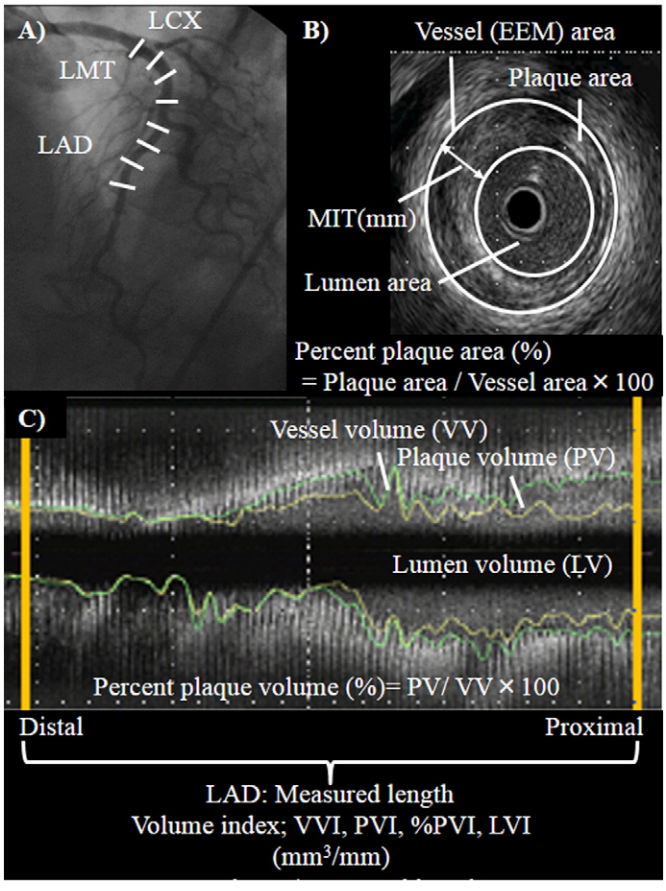

Fig. 2. Definition of volumetric indices in intravascular ultrasound (IVUS) analyses. (A) Angiography; (B) Two-dimensional IVUS analyses; (C) Three-dimensional IVUS analyses. EEM = external elastic membrane; LAD = left anterior descending artery; LCX = left circumflex artery; LMT = left main trunk; LVI = lumen volume index; MIT = maximal intimal thickness; %PVI = percent plaque volume index; PVI = plaque volume; VVI = vessel volume index.

Table 1
Clinical characteristics, medications and laboratory data at study entry.

Variable	EVL group, n = 24	MMF group, n = 39	p value
Donor age, years	41.0 ± 15.7	35.1 ± 12.5	0.130
Male donor, n (%)	14 (70.0)	18 (52.9)	0.218
Recipient age, years	36.5 (26.2–46.4)	33.9 (21.7–44.3)	0.581
Male recipient, n (%)	20 (83.3)	28 (71.8)	0.296
Time since HTx, years	2.2 ± 2.3	2.4 ± 2.2	0.614
Time since HTx, years	1.1 (0.3–3.1)	2.9 (0.2–4.0)	0.966
CMV mismatch (D+/R−), n (%)	3 (17.7)	6 (18.8)	0.924
Cold ischemic time, min	214.0 (179.0–231.0)	207.0 (183.0–218.5)	0.462
VAD before HTx, n (%)	22 (91.7)	33 (84.6)	0.414
Primary reason for HTx			
Non-ICM, n (%)	24 (100)	37 (94.9)	0.260
BMI, kg/m^2	21.4 (19.7–23.1)	19.7 (17.8–22.8)	0.240
Past medical history before HTx			
Hypertension, n (%)	2 (8.3)	12 (30.8)	0.038
Hyperlipidemia, n (%)	4 (16.7)	14 (35.9)	0.101
Diabetes mellitus, n (%)	3(12.5)	8 (20.5)	0.416
Prior smoking history, n (%)	10 (41.7)	12 (30.8)	0.378
PRA class I ≥ 10, n (%)	4 (16.7)	6 (15.4)	0.892
PRA class II ≥10, n (%)	0 (0)	0 (0)	1.000
Rejection grade ≥ 2R before study entry, n (%)	1 (4.2)	5 (12.8)	0.256
Medications at study entry			
Cyclosporine, n (%)	2 (8.3)	8 (20.5)	0.199
Tacrolimus, n (%)	22 (91.7)	31 (79.5)	0.199
Calcium channel blocker, n (%)	19 (79.2)	30 (76.9)	0.835
ACE-I/ARB, n (%)	16 (66.7)	28 (71.8)	0.667
Antiplatelet drug, n (%)	20 (83.3)	38 (97.4)	0.044
Laboratory data at study entry			
Triglycerides, mg/dL	109.0 (92.0–133.0)	94.0 (81.0–120.0)	0.067
Total cholesterol, mg/dL	157.4 ± 27.8	161.6 ± 42.2	0.664
LDL cholesterol, mg/dL	79.8 ± 24.8	86.3 ± 36.3	0.443
HDL cholesterol, mg/dL	53.2 ± 15.1	55.8 ± 13.1	0.468
HbA1c, %	5.2 (4.9–5.8)	4.9 (4.5–5.3)	0.019
Serum creatinine, mg/dL	0.96 (0.76–1.42)	0.84 (0.68–0.96)	0.092
eGFR[a], (mL/min/1.73 m^2)	80.6 (50.4–115.9)	96.9 (83.8–124.8)	0.087

Data are expressed as mean ± SD or median (IQR) for continuous values and number of subjects (%) for categorical values.
ACE-I = angiotensin converting enzyme -inhibitor; ARB = angiotensin II receptor blocker; BMI = body mass index; CMV = cytomegalovirus; D+/R- = donor CMV-positive/recipient CMV-negative; Cre = creatinine; eGFR = estimated glomerular filtration rate; EVL = everolimus; HbA1c = hemoglobin A1c; HDL = high density lipoprotein; HTx = heart transplantation; ICM = ischemic cardiomyopathy; IQR = interquartile range; LDL = low density lipoprotein; MDRD = modification of diet in renal disease; MMF = mycophenolate mofetil; PRA = panel reactive antibody; SD = standard deviation; VAD = ventricular assist device.
[a] eGFR was calculated from creatinine value and the age, using the MDRD equation, modified the Japanese coefficient (0.881), as follows; eGFR (mL/min/1.73 m2) = $0.881 * 186 * \text{age}^{-0.203} * \text{Cre}^{-1.154}$ (for males), $=0.881 * 186 * \text{age}^{-0.203} * \text{Cre}^{-1.154} * 0.742$ (for females).

Table 2
Statin therapy.

	EVL group, n = 24	MMF group, n = 39	p value
Statin at study entry, n (%)	22 (91.7)	39 (100.0)	0.067
Strong statin[a], n (%)	13 (54.2)	13 (33.3)	0.103
High-dose statin[b], n (%)	3 (4.2)	10 (25.6)	0.784
Statin at 1-year follow-up, n (%)	24 (100.0)	39 (100.0)	1.000
Strong statin[a], n (%)	18 (75.0)	15 (38.5)	0.005
High-dose statin[b], n (%)	5 (20.8)	14 (35.9)	0.206
Switch to strong statin, n (%)	4 (16.7)	2 (5.1)	0.130
Intensive statin therapy[c], n (%)	6 (25.0)	2 (5.1)	0.021

Data are expressed as number of subjects (%).
[a] Strong statins include atorvastatin, rosuvastatin, and pitavastatin.
[b] High-dose statins include doses of at least 20 mg/day for pravastatin, 20 mg/day for atorvastatin, 5 mg/day for rosuvastatin, and 4 mg/day for pitavastatin.
[c] Intensive statin therapy was defined as: (1) initiating statin therapy in recipients who did not take statin at study entry, (2) increasing the statin dose between study entry and the 1-year follow-up after entry, and (3) switching to a strong statin from pravastatin between study entry and the 1-year follow-up.

lower in the EVL group ($p = 0.005$). At the 1-year follow-up, VVI decreased and PVI increased in the MMF group ($p = 0.002$ and 0.139, respectively), and these changes resulted in a significant increase in %PVI ($p = 0.006$) and decrease in LVI ($p < 0.001$). In contrast, none of the volumetric indices in the EVL group significantly worsened at 1 year after EVL conversion.

With respect to changes in MIT, there was a greater increase in MIT in the MMF group at the 1-year follow-up ($p = 0.039$), while MIT in the EVL group did not change between study entry and the 1-year follow-up (Table 3). The MMF group had a higher incidence of increased MIT (≥0.3 mm) (4.2% in the EVL group vs. 25.6% the MMF group, $p = 0.029$, Fig. 3).

3.4. Logistic regression analysis for the changes in IVUS parameters

Changes in IVUS measurements were treated as categorical variables using the median value for the change in each IVUS measurement. A logistic regression analysis was conducted to determine whether EVL conversion was associated with changes in the volumetric IVUS indices at the 1-year follow-up (Table 4). Univariate logistic regression analysis showed that EVL conversion was associated with a smaller increase in %PVI and a smaller reduction in LVI ($p = 0.032$ and $p = 0.002$, respectively). After adjustment for the recipient's age, sex, strong statin use at the 1-year follow-up, intensive statin therapy, and the time from HTx to study entry, EVL conversion was significantly associated with a smaller increase in %PVI and a smaller reduction in LVI ($p = 0.026$ and $p = 0.005$, respectively, Table 4; Model 1). A multivariable logistic regression analysis including EVL conversion, previous hypertension, antiplatelet use at study entry, and intensive statin therapy revealed that EVL conversion was significantly associated with a smaller increase in %PVI and a smaller decrease in LVI ($p = 0.044$ and $p = 0.017$, respectively, Table 4; Model 2). However, EVL conversion was not associated with an individual change of VVI or PVI in the logistic regression analysis. Regarding the influence of intensive statin therapy on changes in volumetric IVUS parameters (Supplemental Table S1), intensive statin therapy was significantly associated with a smaller decrease in LVI ($p = 0.046$ in univariate analysis and $p = 0.031$ in the multivariate analysis of model 1).

3.5. IVUS measurements in the early and late cohorts

Recipients were divided into two subgroups according to study entry timing (cutoff of 2 years after HTx, as summarized in Table 5). CAV on IVUS was more developed in the EVL group at study entry in each of the cohorts. The EVL group had 3-fold higher levels of PVI and %PVI than those in the MMF group in the early cohort ($p < 0.001$) and 2-fold higher levels in the late cohort (PVI, $p = 0.005$; %PVI, $p < 0.001$). In the early cohort, the MMF group showed a marginal decrease in VVI, resulting in a marginal increase in %PVI and a significant decrease in LVI ($p = 0.035$) by the 1-year follow-up. Significant increases in MIT between study entry and the 1-year follow-up were observed in the MMF group ($p = 0.041$). In contrast, the EVL group did not show worsening volumetric IVUS indices. In the late cohort, the MMF group showed a significant decrease in VVI ($p = 0.015$), resulting in significant increase in %PVI ($p = 0.046$) and a significant decrease in LVI ($p = 0.005$) at the 1-year follow-up, whereas none of the volumetric indices in the EVL group had significantly worsened by 1 year after EVL conversion.

3.6. Influence of EVL conversion for rejection and laboratory data

The incidence of cellular rejection before study entry was comparable between the two groups (Table 1). During the course of the present study, one recipient in the EVL group had Grade 2R rejection after

Table 3
IVUS measurements at study entry and 1-year follow-up.

	EVL group, n = 24	MMF group, n = 39	p value
VVI, mm^3/mm			
Study entry	11.6 ± 3.0	11.8 ± 2.9	0.744
1-Year follow-up	11.4 ± 3.0	11.0 ± 2.8	0.583
p (entry vs. 1 year)	0.509	0.002	
PVI, mm^3/mm			
Study entry	2.5 (1.8–3.4)	0.9 (0.6–1.7)	<0.001
1-Year follow-up	2.4 (1.8–3.7)	0.9 (0.6–2.1)	<0.001
p (entry vs. 1 year)	0.376	0.139	
% PVI, %			
Study entry	20.9 (17.2–27.2)	9.2 (4.8–14.0)	<0.001
1-Year follow-up	22.3 (14.3–29.2)	9.8 (6.2–15.8)	<0.001
p (entry vs. 1 year)	0.407	0.006	
LVI, mm^3/mm			
Study entry	8.2 (7.2–10.3)	10.1 (8.7–12.0)	0.005
1-Year follow-up	8.3 (7.0–11.7)	9.0 (7.7–11.5)	0.203
p (entry vs. 1 year)	0.53	<0.001	
MIT, mm			
Study entry	1.31 (1.00–1.48)	0.56 (0.36–0.87)	<0.001
1-Year follow-up	1.18 (0.94–1.44)	0.71 (0.39–1.10)	<0.001
p (entry vs. 1 year)	0.166	0.039	
Measured segment length, mm			
Study entry	46.3 ± 8.1	45.9 ± 8.9	0.841

Data are expressed as mean ± SD or median (IQR) for continuous values.
EVL = everolimus; IQR = interquartile range; LVI = lumen volume index; MIT = maximal intimal thickness; MMF = mycophenolate mofetil; PVI = plaque volume index; %PVI = percent plaque volume index; SD = standard deviation; VVI = vessel volume index.

conversion to EVL. These rejection episodes were detected on protocol biopsy and were treated with intravenous methylprednisolone. None of the recipients experienced antibody-mediated rejection.

At the 1-year follow-up, lipid profiles were worse in the EVL group (Table 6). Triglycerides values had increased by 23.6% in the EVL group and decreased by 7.4% in the MMF group ($p = 0.001$). Furthermore, the total cholesterol value had increased by 10.4% in the EVL group but had decreased by 2.0% in the MMF group ($p = 0.02$). However, EVL suppressed the deterioration of renal function (serum creatinine value, a decrease of 7.9% in the EVL group vs. an increase of 5.9% in the MMF group, $p = 0.034$; and eGFR value; an increase of 5.8% in the EVL group vs. a decrease of 6.7% in the MMF group, $p = 0.015$).

3.7. Adverse effects

In our study, we included recipients who could continue on EVL for at least 1 year from initiation in the EVL group. One recipient discontinued EVL because of joint pain and mouth ulcers and thereafter resumed MMF. Although two recipients had mild mouth sores and three recipients had leukopenia, these recipients could continue EVL.

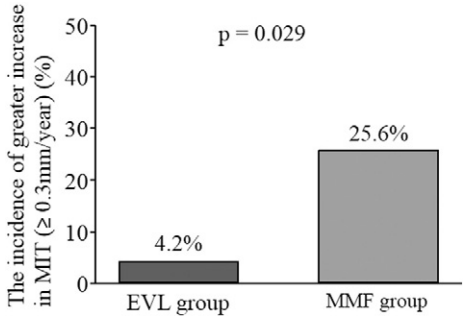

Fig. 3. Comparison of the incidence of greater increases in MIT between the EVL and MMF groups. EVL = everolimus; MIT = maximal intimal thickness; MMF = mycophenolate mofetil.

4. Discussion

We evaluated the efficacy of converting from MMF with standard-dose CNIs to EVL with low-dose CNIs during the maintenance period after HTx. This volumetric IVUS study demonstrated that (1) significant vessel shrinkage and mild increases in plaque volume were observed in the MMF group, resulting in a significant increase in %plaque volume and reduced lumen volume at the 1-year follow-up, whereas these worsening changes in the IVUS indices indicating CAV progression were not observed in the EVL group, and (2) EVL conversion was significantly associated with a smaller increase in percent plaque volume and a smaller decrease in lumen volume. Furthermore, when subjects were divided into sub-groups according to the timing of study entry, (3) the suppressive effect of EVL on the development of CAV was observed in both the early (<2 years after HTx) and late (≥2 years after HTx) conversion groups. These results suggest that EVL conversion reduces changes in IVUS indices that indicate the development of CAV, even when initial CAV has already been developed.

CAV is generally thought to be diffuse and to involve the entire coronary artery [2]. In addition, lumen loss in CAV is caused not only by intimal thickening but also by changes in the external elastic membrane area [23]. As 3D-IVUS versus standard 2D-IVUS analysis can investigate the morphological changes in both the intima and the vessel in the entire coronary artery with high sensitivity and accuracy [24], 3D-IVUS analysis may enable more accurate evaluation of CAV progression.

Whether EVL conversion from MMF attenuates CAV development during maintenance periods after HTx is still controversial [3,5–8]. Arora et al. [8], in a sub-study of 111 patients a mean 5.8 years after HTx who participated in a multicenter, randomized controlled trial with 2D-IVUS, showed that add-on EVL with reduced-dose CNIs did not suppress CAV progression. However, Mancini et al. [3], in a single-center study using 2D-IVUS in 46 subjects with severe CAV who were randomly assigned to receive mTOR inhibitors a mean 3.3 years after HTx, showed that conversion to rapamycin from MMF or AZA attenuated CAV progression. Raichlin et al. [6], in a non-randomized, retrospective study with 3D-IVUS in 69 subjects a mean of 3.8 years after HTx, reported that progression of the plaque index was significantly smaller in the sirolimus group compared to the CNI group. Raichlin's immunosuppressive regimens were conversion to sirolimus with complete CNI withdrawal. Here, our serial 3D-IVUS study demonstrated that EVL conversion a mean 2.3 years after HTx attenuated the changes in IVUS parameters that indicated CAV progression.

Arora et al. [8] showed that the suppressive effects of EVL on CAV progression were different among concomitant immunosuppressants; for example, concomitant AZA with EVL attenuated CAV progression, whereas concomitant MMF with EVL was associated with accelerated CAV. These results suggested the need to evaluate the effects of EVL compared with individual immunosuppressant regimens. Previous studies were limited by the fact that they compared mTOR inhibitors to various immunosuppressants [6–8], whereas our observational study could compare the recipients taking EVL conversion with those with continuing MMF only. In addition, the majority of our recipients took tacrolimus (84.1% of all subjects) instead of cyclosporine. In contrast, EVL was primarily used with cyclosporine in most previous studies [5,6,8]. Therefore, our results also suggest the beneficial effect of concomitant EVL with tacrolimus, not cyclosporine, on slowing the development of CAV.

Although the impact of EVL on morphological changes of CAV remains unclear, experimental studies have reported that mTOR inhibitors affect both vessel remodeling and neointimal proliferation [25,26], which are the key components of CAV progression [23,27]. In our study, the MMF group exhibited plaque progression and vessel shrinkage, resulting in a significant increase in %plaque volume and a significant decrease in lumen volume at the 1-year follow-up. In contrast, the EVL group did not show these worsening changes of IVUS parameters. Although EVL conversion was not associated with the individual

Table 4
Logistic regression analysis for changes in IVUS parameters.

	Odds ratio of conversion to EVL (95% CI)					
	Univariate	p value	Model 1	p value	Model 2	p value
%PVI (cut off ≥0.7%)	0.31 (0.11–0.91)	0.032	0.24 (0.07–0.84)	0.026	0.25 (0.07–0.97)	0.044
LVI (cut off ≥−3.9%)	6.00 (1.92–18.74)	0.002	8.69 (1.94–38.89)	0.005	5.14 (1.34–19.75)	0.017
VVI (cut off ≥−4.3%)	2.88 (0.99–8.31)	0.051	2.82 (0.86–9.27)	0.088	2.35 (0.65–8.42)	0.191
PVI (cut off ≥4.8%)	0.42 (0.15–1.19)	0.101	0.31 (0.09–1.04)	0.058	0.43 (0.12–1.55)	0.197

Model 1: adjusted for recipient age, sex, strong statin use at 1-year follow-up, intensive statin therapy and the time from heart transplantation to study entry.
Model 2: Multivariable logistic regression including variables with $P < 0.05$ in comparison between 2 groups as shown Table 1. (EVL conversion, previous hypertension, antiplatelet use, HbA1c level, and intensive statin therapy).
CI = confidence interval; EVL = everolimus; HbA1c = hemoglobin A1c; IVUS = intravascular ultrasound; LVI = lumen volume index; PVI = plaque volume index; %PVI = percent plaque volume index; VVI = vessel volume index.

changes in vessel and plaque volumes, the sum of these changes resulted in a significant difference of changes in lumen volume and %plaque volume. This result suggests that the impact of EVL conversion on preventing both negative vessel remodeling and intimal proliferation contributes to the suppressive effect on CAV progression. The mechanisms of constrictive versus expansive remodeling are not well known [28,29], but the ability to undergo compensatory vessel enlargement in response to plaque growth has been reported to be dependent on coronary endothelial cell or smooth muscle cell function [30]. Previous studies have shown that mTOR inhibitor vs. cyclosporine use was associated with preserved coronary endothelial function and improved vascular smooth muscle cell function in the transplanted artery [25,30,31]. The beneficial effects of EVL on vessel remodeling in CAV may be one mechanism by which EVL inhibits CAV progression in HTx recipients in the maintenance period. Additionally, Potena et al. showed that a greater increase in MIT (>0.35 mm) 1 to 5 years post-HTx was associated with poor clinical outcomes more than 5 years after post-HTx [19]. Based on our results that the MMF group exhibited a greater incidence of a larger increase in MIT (MIT ≥0.3 mm at the 1-year follow-up) than did the EVL group, EVL conversion from MMF may contribute to improvements in long-term outcome after HTx.

The appropriate timing of conversion to EVL has not yet been established [3,5,8,10,11]. Since previous serial IVUS studies have shown that CAV progresses faster in the early periods and later slows [32–34], early initiation of EVL has been thought to be more effective for preventing CAV progression compared with late initiation. Our study demonstrated that late conversion (≥2 years after HTx) attenuated CAV development as well as early conversion (<2 years after HTx). In contrast, a recent retrospective study of 3D-IVUS in 52 recipients by Masetti et al. [5] showed a conflicting result, in that late conversion to EVL did not attenuate CAV progression compared with MMF, whereas early EVL conversion did. They described that EVL exerted preventive effects on CAV progression by antagonizing immune-mediated endothelial cell injury, and EVL's efficacy on CAV suppression might weaken in the late periods after HTx because metabolic risk factors play a more relevant role in favoring late CAV progression rather than immune-mediated action. The exact mechanisms of the time-dependent effects of mTOR inhibitors remain unclear. Importantly, a mechanism by which mTOR inhibitors attenuate CAV development has been explained not only by a reduction in T-cell-mediated immunity but also by anti-proliferative effects on vascular smooth muscle cell growth [27]. Proliferation of smooth muscle and endothelial cells was induced by both immune- and non-immune response mediated injury to endothelial cells in the transplanted coronary artery [26,27]. Such proliferation is known to be the final common pathway in intimal hyperplasia, independently of the cause of endothelial cell injury [27]. Therefore, EVL's

Table 5
IVUS measurements in sub-divided groups according to the study entry time from heart transplantation.

	Early <2 years			Late ≥2 years		
	EVL group n = 15	MMF group n = 18	p value	EVL group n = 9	MMF group n = 21	p value
Time since HTx, year	0.9 (0.1–1.0)	0.2 (0.1–1.0)	0.023	4.0 (3.0–4.6)	3.9 (3.0–5.0)	0.635
VVI, mm^3/mm						
Study entry	12.0 ± 3.3	12.6 ± 3.3	0.656	10.8 ± 2.6	11.2 ± 2.5	0.689
1-Year follow-up	11.8 ± 3.2	11.8 ± 3.4	0.984	10.8 ± 2.6	10.4 ± 2.0	0.622
p (entry vs. 1 year)	0.521	0.062		0.908	0.015	
PVI, mm^3/mm						
Study entry	2.5 (1.9–3.5)	0.8 (0.6–1.6)	0.001	2.0 (1.7–3.2)	1.0 (0.6–1.7)	0.005
1-Year follow-up	3.0 (2.1–3.9)	0.6 (0.59–1.6)	0.008	1.9 (1.8–2.3)	1.0 (0.8–2.3)	0.067
p (entry vs. 1 year)	0.999	0.199		0.214	0.414	
% PVI						
Study entry	21.3 (15.4–26.6)	7.4 (4.7–10.9)	<0.001	20.1 (19.0–27.8)	10.6 (5.8–15.4)	<0.001
1-Year follow-up	23.7 (15.8–37.2)	8.5 (5.0–11.1)	0.002	18.4 (12.7–26.0)	13.5 (7.6–18.1)	0.060
p (entry vs. 1 year)	0.955	0.071		0.173	0.046	
LVI, mm^3/mm						
Study entry	8.5 (6.9–11.8)	10.8 (8.8–13.3)	0.047	7.5 (7.3–8.4)	10.0 (8.6–10.6)	0.013
1-Year follow-up	8.6 (7.1–11.8)	9.2 (8.0–12.5)	0.159	8.2 (7.0–8.5)	8.7 (7.7–10.1)	0.483
p (entry vs. 1 year)	0.91	0.035		0.214	0.005	
MIT, mm						
Study entry	1.3 (1.1–1.5)	0.4 (0.3–0.7)	<0.001	1.3 (0.9–1.4)	0.6 (0.4–1.0)	0.011
1-Year follow-up	1.3 (1.1–1.5)	0.6 (0.4–0.9)	0.005	1.0 (0.9–1.2)	0.8 (0.5–1.2)	0.161
P (entry vs 1 year)	0.514	0.041		0.138	0.313	
Measured segment length, mm						
Study entry	44.3 ± 9.8	45.8 ± 9.4	0.661	49.7 ± 1.0	45.9 ± 8.7	0.216

Data are expressed as mean ± SD or median (IQR) for continuous values.
EVL = everolimus; IQR = interquartile range; LVI = lumen volume index; MIT = maximal intimal thickness; MMF = mycophenolate mofetil; PVI = plaque volume index; %PVI = percent plaque volume index; SD = standard deviation; VVI = vessel volume index.

Table 6
The percent change of laboratory data at 1-year follow-up.

Variable	EVL group, n = 24	MMF group, n = 39	p value
Triglycerides, %	23.6 (−0.7–54.9)	−7.4 (−34.1–6.4)	0.001
Total cholesterol, %	10.4 ± 20.8	−2.0 ± 19.6	0.020
LDL cholesterol, %	13.0 ± 39.2	−3.7 ± 32.0	0.069
HDL cholesterol, %	−2.2 (−20.1–16.5)	6.7 (−8.5–23.8)	0.322
HbA1c, %	8.2 ± 10.8	7.4 ± 10.9	0.773
Serum creatinine, %	−7.9 (−14.5–12.4)	5.9 (−3.6–15.9)	0.034
eGFR[a], %	5.8 ± 19.2	−6.7 ± 19.6	0.015

Data are expressed as mean ± SD, median (IQR).
Cre = creatinine; eGFR = estimated glomerular filtration rate; EVL = everolimus; HbA1c = hemoglobin A1c; HDL = high density lipoprotein; IQR = interquartile range; LDL = low density lipoprotein; MDRD = modification of diet in renal disease; MMF = mycophenolate mofetil; SD = standard deviation.
[a] eGFR (mL/min/1.73 m^2) was calculated from creatinine value and the age, using the MDRD equation, modified the Japanese coefficient (0.881), as follows; eGFR (mL/min/1.73 m^2) = $0.881 * 186 * age^{-0.203} * Cre^{-1.154}$ (for males), = $0.881 * 186 * age^{-0.203} * Cre^{-1.154} * 0.742$ (for females).

anti-proliferative effects may mediate the suppressive effects on CAV development independently of the timing of conversion. Another potential reason for our discordant results compared with previous results on late EVL conversion may be merely due to a bias of the larger differences in initial IVUS indices between the two groups. However, this bias would generally lead us to underestimate the impact of EVL on CAV progression.

In the present study, EVL conversion with reduced CNI did not increase acute rejection and the deterioration of renal function. When mTOR inhibitors are added with CNI, the toxic effects of CNI increase [35]. Therefore, the use of mTOR inhibitors without CNIs has recently emerged as an alternative therapeutic option for recipients with CNI-induced nephropathy [6,7]. However, since one study reported that the incidence of acute rejection was higher in recipients undergoing conversion to SRL without CNIs [36], whether mTOR inhibitors with CNI-free regimens is superior to mTOR inhibitors with reduced-dose CNIs remains unclear. Our results also may provide evidence that EVL conversion with a low-dose CNI regimen is feasible and safe for recipients with renal dysfunction.

The frequency of adverse effects of EVL was not high. On the other hand, similar to the findings of previous studies [5,6], our results showed that the lipid profiles in recipients were worsened.

5. Study limitations

The main limitations of this study were the small sample size and the observational, retrospective design without randomization. Because of this observational design, an indication bias could not be avoided. Since one of the main reason for EVL conversion was the early development of CAV as shown on IVUS, the incidence of CAV at study entry was higher in the EVL group than in the MMF group. Therefore, we could not fully compare the changes in IVUS indices between the EVL and MMF groups. Finally, although CAV progression is known to be affected by donor factors, we could not adjust for these variables in the multivariable model because of missing of donor information for recipients who underwent HTx in other countries.

6. Conclusion

Our retrospective, observational study showed that conversion from MMF with standard-dose CNIs to EVL with low-dose CNIs may reduce the worsening change for IVUS indices that indicate CAV development. Additionally, our results indicate that late conversion to EVL also may reduce the worsening changes of IVUS indices. These results suggest that EVL conversion is effective for slowing CAV progression when CAV has already developed. In addition, this 3D-IVUS study suggests that the suppressive effects of EVL on CAV progression may be induced not only by reducing plaque progression but also by suppressing vessel shrinkage.

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Conflicts of interest

None.

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References

[1] L.H. Lund, L.B. Edwards, A.Y. Kucheryavaya, A.I. Dipchand, C. Benden, J.D. Christie, et al., International society for heart and lung transplantation, the registry of the international society for heart and lung transplantation: thirtieth official adult heart transplant report-2013; focus theme: age, J. Heart Lung Transplant. 32 (2013) 951–964.
[2] M. Rahmani, R.P. Cruz, D.J. Granville, B.M. McManus, Allograft vasculopathy versus atherosclerosis, Circ. Res. 99 (2006) 801–815.
[3] D. Mancini, S. Pinney, D. Burkhoff, J. LaManca, S. Itescu, E. Burke, et al., Use of rapamycin slows progression of cardiac transplantation vasculopathy, Circulation 108 (2003) 48–53.
[4] J.H. Eisen, E.M. Tuzcu, R. Dorent, J. Kobashigawa, D. Mancini, H.A. Valantine-von Kaeppler, et al., RAD B253 study group, everolimus for the prevention of allograft rejection and vasculopathy in cardiac transplantation recipients, N. Engl. J. Med. 349 (2003) 847–858.
[5] M. Masetti, L. Potena, M. Nardozza, P. Prestinenzi, N. Taglieri, F. Saia, et al., Differential effect of everolimus on progression of early and late cardiac allograft vasculopathy in current clinical practice, Am. J. Transplant. 13 (2013) 1217–1226.
[6] E. Raichlin, J.H. Bae, Z. Khalpey, B.S. Edwards, W.K. Kremers, A.L. Clavell, et al., Conversion to sirolimus as primary immunosuppression attenuates the progression on allograft vasculopathy after cardiac transplantation, Circulation 116 (2007) 2726–2733.
[7] Y. Topilsky, T. Hasin, E. Raichlin, B.A. Boilson, J.A. Schirger, N.L. Pereira, et al., Sirolimus as primary immunosuppression attenuates allograft vasculopathy with improved late survival and decreased cardiac events after cardiac transplantation, Circulation 125 (2012) 708–720.
[8] S. Arora, T. Ueland, B. Wennerblom, V. Sigurdadottir, H. Eiskjær, H.E. Bøtker, et al., Effect of everolimus introduction on cardiac allograft vasculopathy–results of randomized, multicenter trial, Transplantation 92 (2011) 235–243.
[9] J. Kobashigawa, L. Miller, D. Renlund, R. Mentzer, E. Alderman, R. Bourge, et al., A randomized active-controlled trial of mycophenolate mofetil in heart transplant recipients, Transplantation 66 (1998) 507–515.
[10] J.A. Kobashigawa, D.F. Pauly, R.C. Starling, H. Eisen, H. Ross, S.S. Wang, et al., A2310 IVUS substudy investigators, cardiac allograft vasculopathy by intravascular ultrasound in heart transplant patients, JACC Heart Fail. 1 (2013) 389–399.
[11] H.J. Eisen, J. Kobashigawa, R.C. Strarling, D.F. Pauly, A. Kfoury, H. Ross, et al., Everolimus versus mycophenolate mofetil in heart transplantation: a randomized, multicenter trial, Am. J. Transplant. 13 (2013) 1203–1216.
[12] T. Nakatani, Heart transplantation, Circ. J. 73 (Suppl. A) (2009) A55–A60.
[13] K. Wada, M. Takada, T. Kotake, H. Ochi, H. Morishita, K. Komamura, et al., Limited sampling strategy for mycophenolic acid in Japanese heart transplant recipients: comparison of cyclosporin and tacrolimus treatment, Circ. J. 71 (2007) 1022–1028.
[14] D.A. Heimansohn, R.J. Robison, J.M. Paris, R.G. Matheny, J. Bogdon, C.J. Shaar, Routine surveillance endomyocardial biopsy: late rejection after heart transplantation, Ann. Thorac. Surg. 64 (1997) 1231–1236.
[15] S. Stewart, G.L. Winters, M.C. Fishbein, H.D. Tazelaar, J. Kobashigawa, J. Abrams, et al., Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection, J. Heart Lung Transplant. 24 (2005) 1710–1720.
[16] M.R. Mehra, M.G. Crespo-Leiro, A. Dipochand, S.M. Ensminger, N.E. Hiemann, J.A. Kobashigawa, et al., International society for heart and lung transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010, J. Heart Lung Transplant. 29 (2010) 717–727.
[17] J.A. Kobashigawa, J.M. Tobis, R.C. Starling, E.M. Tuzcu, A.L. Smith, H.A. Valantine, et al., Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years, J. Am. Coll. Cardiol. 45 (2005) 1532–1537.
[18] E.M. Tuzcu, S.R. Kapadia, R. Sachar, K.M. Ziada, T.D. Crowe, J. Feng, et al., Intravascular ultrasound evidence of angiographically silent progression in coronary atherosclerosis predicts long-term morbidity and mortality after cardiac transplantation, J. Am. Coll. Cardiol. 45 (2005) 1538–1542.
[19] L. Potena, M. Masetti, M. Sabatino, M.L. Bacchi-Reggiani, V. Pece, P. Prestinenzi, et al., Interplay of coronary angiography and intravascular ultrasound in predicting long-

term outcomes after heart transplantation, J. Heart Lung Transplant. 34 (2015) 1146–1153.
[20] T. Nakatani, N. Fukushima, M. Ono, Y. Saiki, H. Matsuda, R. Yozu, et al., The registry report of heart transplantation in Japan, Circ. J. 78 (2014) 2604–2609.
[21] J.A. Kobashigawa, S. Katznelson, H. Laks, J.A. Johnson, L. Yeatman, X.M. Wang, et al., Effect of pravastatin on outcomes after cardiac transplantation, N. Engl. J. Med. 333 (1995) 621–627.
[22] E. Imai, M. Horio, K. Nitta, K. Yamagata, K. Iseki, S. Hara, et al., Estimation of glomerular filtration rate by the mdrd study equation modified for Japanese patients with chronic kidney disease, Clin. Exp. Nephrol. 11 (2007) 41–50.
[23] K. Pethig, B. Heublein, T. Wahlers, A. Haverich, Mechanism of luminal narrowing in cardiac allograft vasculopathy: inadequate vascular remodeling rather than intimal hyperplasia is the major predictor of coronary artery stenosis. Working group on cardiac allograft vasculopathy, Am. Heart J. 135 (1998) 628–633.
[24] G.S. Mintz, S.E. Nissen, W.D. Anderson, S.R. Bailey, R. Erbel, P.J. Fitzgerald, et al., American college of cardiology clinical expert consensus document on standards for acquisition, measurement and reporting of intravascular ultrasound studies (IVUS). A report of the American college of cardiology task force on clinical expert consensus documents, J. Am. Coll. Cardiol. 37 (2001) 1478–1492.
[25] D. Ramzy, V. Rao, L.C. Tumiati, N. Xu, S. Miriuka, D. Delgado, et al., Role of endothelin-1 and nitric oxide bioavailability in transplant-related vascular injury: comparative effects of rapamycin and cyclosporine, Circulation 114 (1 Suppl.) (2006) I214–I219.
[26] S.O. Marx, T. Jayaraman, L.O. Go, A.R. Marks, Rapamycin–FKBP inhibits cell cycle regulators of proliferation in vascular smooth muscle cells, Circ. Res. 76 (1995) 412–417.
[27] R.S. Poston, M. Billingham, G. Hoyt, J. Pollard, R. Shorthouse, R.E. Morris, et al., Rapamycin reverses chronic graft vascular disease in a novel cardiac allograft model, Circulation 100 (1999) 67–74.
[28] S. Glagov, E. Weisenberg, C.K. Zarins, R. Stankunavicius, G.J. Kolettis, Compensatory enlargement of human atherosclerotic coronary arteries, N. Engl. J. Med. 316 (1987) 1371–1375.
[29] A. Jeremias, C. Spies, N.A. Herity, M.R. Ward, E. Pomerantsev, P.G. Yock, et al., Coronary distensibility and compensatory vessel enlargement — a novel parameter influencing vascular remodeling? Basic Res. Cardiol. 96 (2001) 506–512.
[30] E. Raichlin, A. Prasad, W.K. Kremers, B.S. Edwards, C.S. Rihal, A. Lerman, et al., Sirolimus as primary immunosuppression is associated with improved coronary vasomotor function compared with calcineurin inhibitors in stable cardiac transplant recipients, Eur. Heart J. 30 (2009) 1356–1363.
[31] S.S. Sinha, M.X. Pham, R.H. Vagelos, M.G. Perlroth, S.A. Hunt, D.P. Lee, et al., Effect of rapamycin therapy on coronary artery physiology early after cardiac transplantation, Am. Heart J. 155 (2008) e1–e6 (889).
[32] H. Tsutsui, K.M. Ziada, P. Schoenhagen, A. Iyisoy, W.A. Magyar, T.D. Crowe, et al., Lumen loss in transplant coronary artery disease is a biphasic process involving early intimal thickening and late constrictive remodeling: results from a 5-year serial intravascular ultrasound study, Circulation 104 (2001) 653–657.
[33] K. Pethig, B. Heublein, R.R. Meliss, A. Haverich, Volumetric remodeling of the proximal left coronary artery: early versus late after heart transplantation, J. Am. Coll. Cardiol. 34 (1999) 197–203.
[34] J.A. Kobashigawa, L. Wener, J. Johnson, J.W. Currier, L. Yeatman, J. Cassem, et al., Longitudinal study of vascular remodeling in coronary arteries after heart transplantation, J. Heart Lung Transplant. 19 (2000) 546–550.
[35] H. Podder, S.M. Stepkowski, K.L. Napoli, J. Clark, R.R. Verani, T.C. Chou, et al., Pharmacokinetic interactions augument toxicities of sirolimus/cyclosporine combinations, J. Am. Soc. Nephrol. 12 (2001) 1059–1071.
[36] A. Zuckermann, A. Keogh, M.G. Crespo-Leiro, D. Mancini, F.G. Vilchez, L. Almenar, et al., Randomized controlled trial of sirolimus conversion in cardiac transplant recipients with renal insufficiency, Am. J. Transplant. 12 (2012) 2487–2497.