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Attenuation of cardiac allograft vasculopathy by sirolimus: Relationship to time interval after heart transplantation

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KEYWORDS:
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calcineurin inhibitors;
plaque composition;
plaque progression

BACKGROUND: The aim of the study was to assess temporal changes in plaque size and components after heart transplantation (HTx), and to evaluate the differences in treatment effects on plaque progression between sirolimus and calcineurin inhibitors (CNIs).

METHODS: The study comprised 146 HTx recipients who were converted from CNIs to sirolimus as primary immunosuppressant (sirolimus group, $n = 61$) and those who were maintained on CNIs (CNI group, $n = 85$). A retrospective compositional analysis of serial virtual histology–intravascular ultrasound was performed.

RESULTS: During a median follow-up of 2.8 years, there was a significant difference in plaque volume in favor of sirolimus between groups ($p = 0.004$). When subjects were sub-classified according to the time interval between HTx and study inclusion, those in the early group (≤2 years after HTx) had a greater increase in plaque volume ($p = 0.006$), characterized by a higher progression rate of fibrous plaque volume ($p = 0.01$). The treatment difference between groups in plaque volume was identified in the early group in favor of sirolimus with attenuating effects on the progression of fibrous plaque component (both $p = 0.03$ for interaction). By contrast, there were significant differences in necrotic core and dense calcium volume (both $p < 0.05$ for interaction) in favor of CNIs in the late group (≥6 years after HTx).

CONCLUSIONS: Compared with continued CNI therapy, sirolimus attenuated plaque progression in recipients with early conversion, but contributed to increases in necrotic core and dense calcium volume in those with late conversion. Our study supports the hypothesis that early initiation of sirolimus offers greater benefits in the treatment of CAV.

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Although major improvements have been made in the prevention and treatment of acute transplant rejection, cardiac allograft vasculopathy (CAV) remains one of the most important factors limiting long-term survival in heart transplantation (HTx). Calcineurin inhibitors (CNIs) such as cyclosporine and tacrolimus, which have been pivotal in reducing acute rejection and improving early survival, are widely used for primary immunosuppressants of de novo HTx.[1] Recently, the use of proliferation signal inhibitors (PSIs), such as sirolimus and its derivative, everolimus, in combination with a reduction or withdrawal of CNIs, has emerged as an alternative therapeutic option for maintenance therapy in patients with CNI intolerance,

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CNI-induced nephropathy, or CAV development. In an effort to minimize the potential of renal dysfunction due to the combined use of sirolimus and CNI,[2,3] much attention has been directed toward a CNI-free regimen.

Previous intravascular ultrasound studies from our group demonstrated that conversion to CNI-free, sirolimus-based immunosuppression was associated with attenuation of development of CAV[4,5] and a reduced incidence of future cardiac events,[6] even in patients initially receiving a CNI after HTx.

Little is known about the effects of sirolimus on plaque morphology in CAV over time. Also, not much is known about the natural history of CAV lesions in terms of plaque characteristics. The aim of this study was to quantify changes in plaque size and components over time after HTx, and to evaluate the differences in treatment effects on plaque progression between sirolimus and CNIs.

Methods

Study protocol

In the HTx program at the Mayo Clinic, virtual histology™ intravascular ultrasound (VH-IVUS) of the left anterior descending coronary artery (LAD) has been performed since 2005 in HTx recipients in conjunction with routine annual coronary angiography for surveillance of CAV. We selected the following groups of patients for conversion from CNI to sirolimus as the primary immunosuppressant: those impaired renal function likely secondary to CNIs (glomerular filtration rate ≤50 ml/min without any other identifiable cause of renal dysfunction); those with CAV (any epicardial coronary artery stenosis ≥50% in any major branch and/or distal pruning of secondary side branches) detected on annual coronary angiography; those with severe CNI-related side effects; and those with an interest in conversion 3 to 6 months after transplantation based on our routine conversion protocol, as previously described.[4–6]

In this retrospective study of a clinical practice change, a total of 214 HTx recipients were initially screened for inclusion. All patients underwent VH-IVUS examination within 1 year after conversion from CNI to sirolimus (sirolimus group), or underwent VH-IVUS, for those maintained on CNI (CNI group), between June 2005 and December 2010 (Figure 1).

Of the 214 patients, 41 without follow-up VH-IVUS (23 had renal dysfunction, 7 were chosen at the discretion of treating physicians, 6 did not complete 1-year follow-up, 3 died before the second IVUS, 1 had limited arterial access, and 1 was followed-up elsewhere) were not included in the study. The following subjects were also excluded from the analysis: (1) patients converted back to CNI before follow-up IVUS in the sirolimus group ($n = 9$); (2) patients treated with both sirolimus and CNI for prior episodes of rejection ($n = 6$); (3) patients undergoing coronary intervention of the LAD ($n = 8$); and (4) patients with inadequate IVUS images limiting quantitative analysis ($n = 4$). Thus, a total of 146 patients were included in the analyses. For patients with more than 2 IVUS evaluations during the study period, we included the first (baseline) and the last (follow-up) IVUS examinations in the analysis.

In a further sub-analysis, 1-year follow-up IVUS data, which were available in 99 of 146 patients, were used to assess changes in plaque volume during the 1-year observation period between 2 treatment groups. All transplant recipients received induction therapy with low-dose OKT3 or anti-thymocyte globulin as part of a standard induction protocol. Sirolimus, cyclosporine, tacrolimus, prednisone, azathioprine and mycophenolate mofetil were managed and dosed as described elsewhere.[5] Second immunosuppressive agents, mycophenolate mofetil or azathioprine, were left unchanged during follow-up, as was the existing dose of prednisone. To focus on the optimal timing of conversion to sirolimus, study subjects were sub-classified into 3 groups according to the time interval between HTx and study inclusion (baseline IVUS): early (≤2 years, $n = 52$ [16 sirolimus, 36 CNI]); intermediate (between 3 and 5 years, $n = 44$ [24 sirolimus, 20 CNI]); and late (≥6 years, $n = 50$ [21 sirolimus, 29 CNI]). Data on patient characteristics were collected before each IVUS examination. The study was approved by the institutional review board of the Mayo Clinic and all patients provided written informed consent to participate.

Coronary angiography

Based on the International Society for Heart and Lung Transplantation (ISHLT) guidelines,[7] CAV was classified by coronary angiography as: ISHLT CAV 0 (not significant); CAV 1 (mild); CAV 2 (moderate); and CAV 3 (severe).

Gray-scale and VH-IVUS image acquisition and analysis

IVUS images were acquired after administration of 100 to 200 μg of intracoronary nitroglycerin with a phased-array, 20-MHz, 3.2F IVUS imaging catheter (Eagle Eye Gold; Volcano Therapeutics, Inc., Rancho Cordova, CA). The IVUS catheter was placed distally by using a fiduciary side branch as the starting point, and a motorized pull-back was performed at 0.5 mm/s up to the LAD ostium. During pull-back, the electrocardiographic (ECG)-gated gray-scale IVUS images were acquired and radiofrequency data were captured at the top of the R-wave.

Off-line volumetric reconstruction was performed using pcVH (version 2.2) or Volcano Image Analysis software (version 3.1;

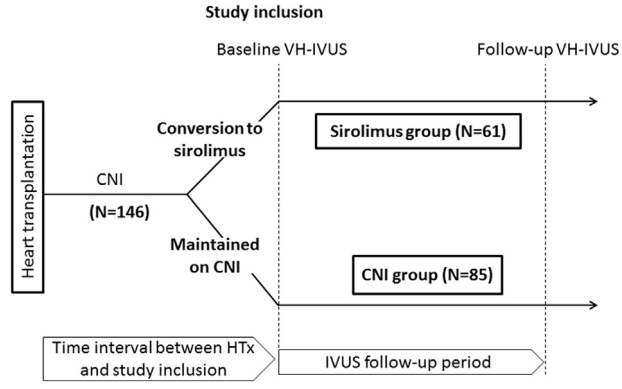

Figure 1 Time-line of our study. In this retrospective study of a clinical practice change, the sirolimus group consisted of patients undergoing IVUS examination within 1 year after conversion from CNI to sirolimus. The CNI group comprised those who were maintained on CNI and underwent IVUS examinations during the study period. The first (baseline) and the last (follow-up) IVUS examinations were used for analysis. Study patients were sub-classified into 3 groups according to the time interval between heart transplantation and study inclusion (baseline IVUS): early (≤2 years); intermediate (between 3 to 5 years); or late (≥6 years). CNI indicates calcineurin inhibitor; HTx, heart transplantation; IVUS, intravascular ultrasound.

Table 1 Patients' Characteristics

	Sirolimus ($N = 61$)	CNI ($N = 85$)	p
Recipient age (years)	56 ± 11	53 ± 14	0.16
Male gender [*n* (%)]	43 (70)	65 (76)	0.42
Time since heart transplant (years)	2.5 (1.2,7.0)	2.9 (0.2,8.5)	0.38
Donor age (years)	29 ± 13	30 ± 13	0.82
Previous CMV infection [*n* (%)]	17 (28)	22 (26)	0.79
Treatment episodes of rejection [*n* (%)]			
Baseline	10 (16)	13 (15)	0.86
During follow-up period	2 (3)	7 (8)	0.22
Comorbidities			
BMI (kg/m^2)			
Baseline	27.5 ± 5.1	26.9 ± 5.5	0.52
Follow-up	28.3 ± 5.8[a]	27.6 ± 5.3[a]	0.45
Diabetes [*n* (%)]			
Baseline	14 (23)	19 (22)	0.93
Follow-up	14 (23)	19 (22)	0.93
Hypertension [*n* (%)]			
Baseline	48 (79)	58 (68)	0.16
Follow-up	47 (77)	57 (67)	0.19
Total cholesterol (mg/dl)			
Baseline	198 ± 46	184 ± 47	0.07
Follow-up	194 ± 57	177 ± 48	0.06
Triglyceride (mg/dl)			
Baseline	148 (114,222)	128 (87,193)	0.06
Follow-up	168 (120,226)	135 (86,191)	0.01
HDL cholesterol (mg/dl)			
Baseline	55 ± 17	54 ± 18	0.59
Follow-up	50 ± 16[b]	52 ± 14	0.36
LDL cholesterol (mg/dl)			
Baseline	109 ± 34	100 ± 35	0.12
Follow-up	100 ± 30	93 ± 37	0.26
Serum creatinine (mg/dl)			
Baseline	1.4 ± 0.6	1.4 ± 0.3	0.94
Follow-up	1.2 ± 0.4[a]	1.4 ± 0.6	0.04
Medication			
Aspirin [*n* (%)]			
Baseline	17 (28)	23 (27)	0.91
Follow-up	19 (31)	25 (29)	0.82
ACE inhibitor [*n* (%)]			
Baseline	25 (41)	29 (34)	0.40
Follow-up	24 (39)	30 (35)	0.61
CCB [*n* (%)]			
Baseline	13 (21)	26 (31)	0.21
Follow-up	14 (23)	28 (33)	0.19
Statin [*n* (%)]			
Baseline	56 (92)	72 (85)	0.19
Follow-up	57 (93)	75 (88)	0.29
Immunosuppressants			
Sirolimus [*n* (%)]	61 (100)	–	
Cyclosporine [*n* (%)]	–	61 (72)	
Tacrolimus [*n* (%)]	–	24 (28)	
Azathioprine [*n* (%)]	25 (41)	25 (29)	0.15
MMF [*n* (%)]	35 (57)	57 (67)	0.23

Data are expressed as mean ± SD, median (interquartile range) or number (%). ACE, angiotensin-converting enzyme; BMI, body mass index; CCB, calcium channel blocker; CMV, cytomegalovirus; CNI, calcineurin inhibitors; HDL, high-density lipoprotein; IVUS, intravascular ultrasound; LDL, low-density lipoprotein; MMF, mycophenolate mofetil.

[a] $p < 0.05$

[b] $p < 0.001$ vs baseline.

Volcano Corp.) by 2 experienced observers blinded to baseline the patients' characteristics. Three or 4 matched coronary segments of the LAD were determined from the images acquired at baseline and follow-up studies on the basis of the fiducial location of distal and proximal major side branches. The length of the segment was assessed as the distance between these 2 side branches. For each coronary segment, vessel (external elastic membrane) and lumen borders were contoured for all recorded frames. Quantitative IVUS measurements included vessel volume, lumen volume and plaque (vessel − lumen) volume and plaque burden (plaque volume / vessel volume × 100). Radiofrequency IVUS plaque components were color-coded and reported as absolute plaque volume of VH-IVUS parameters (fibrous [dark green], fibrofatty tissue [light green], necrotic core [red], dense calcium [white]).[8] All volumetric data were divided by segment length to compensate for the different segment length of each examined artery and were shown as a volume index (mm^3/mm). The segment with the largest change in plaque volume index from baseline to follow-up at any matched site was used for the analysis in each patient. In a previous report we assessed interobserver variability for VH compositional data in transplant recipients.[9]

Statistical analysis

Variables were expressed as mean ± SD, median (interquartile range [IQR]) or count (percentage). Differences in baseline parameters were analyzed by Student's *t*-test, Mann–Whitney *U*-test, chi-square test or 1-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test when appropriate. Within-treatment-group changes from baseline were evaluated by paired *t*-tests. Changes from baseline in IVUS parameters, which were divided by follow-up periods (years) to compensate for the different time interval between 2 IVUS examinations, were analyzed using an analysis of covariance (ANCOVA) model with treatment as a major factor and baseline value as a covariate. Treatment differences were summarized as the difference between treatment groups (sirolimus minus CNI) in adjusted mean, 95% confidence interval (CI) and *p*-value. In the subgroup analysis, the interaction between subgroup and treatment (sirolimus or CNI) was tested with the interaction test of 2-way ANCOVA, and treatment effects were assessed by the ANCOVA model including a subgroup-allocated treatment interaction term. Likewise, treatment differences were assessed between 2 IVUS examinations performed at baseline and at 1-year follow-up by the ANCOVA model. To account for confounding due to differences in time since heart transplant between 2 treatment groups at baseline for changes in plaque volume (mm^3/mm/year), multivariate linear regression analyses were performed, adjusting for treatment groups, time since heart transplant and baseline plaque volume index. All statistical analyses were performed with JMP, version 9.0 (SAS Institute, Inc., Cary, NC). $p < 0.05$ was considered significant.

Results

Baseline characteristics

Of 146 recipients, 61 were converted to sirolimus at a median of 2.5 (1.2 to 7.0) years after HTx. The reasons for conversion were CNI-induced nephropathy in 35 patients, CAV detected on annual angiography in 5, CNI-related side effects in 3, and our routine conversion protocol in 18. Baseline characteristics were comparable between the 2 groups (Table 1). Cholesterol and triglyceride levels were slightly but non-significantly higher in patients treated with sirolimus. Concurrent medications were administered to patients in the sirolimus and CNI groups. On coronary angiography, 43 (70%) subjects were graded CAV 0, 16 (26%) CAV 1, 1 (2%) CAV 2 and 1 (2%) CAV 3 in the sirolimus group, whereas 48 (56%) were CAV 0, 33 (39%) CAV 1, 4 (5%) CAV 2 and 0 (0%) CAV 3 in the CNI group ($p = 0.16$).

IVUS measurements

Table 2 shows baseline and follow-up results of IVUS analyses in all study subjects.<TBL ID = 2> When the change in plaque volume index from baseline was compared between treatment groups, there was a significant treatment effect in favor of sirolimus (least-squares mean [sirolimus minus CNI] −0.36 mm^3/mm/year, 95% CI −0.60 to −0.12; $p = 0.004$). In the VH-IVUS analyses, the comparison of change from baseline between treatment groups did not show any significant differences.

Time course of plaque development after HTx

Figure 2 shows differences in plaque progression rate among the 3 groups stratified according to the time interval between HTx and study inclusion in all subjects. Patients' characteristics among the 3 study arms are shown in Table 3. The average annual changes in plaque volume were different over time among the 3 study arms ($p = 0.006$; Figure 2A). Recipients in the early group had a greater rate of increase in plaque volume than those in the intermediate group ($p < 0.01$). VH-IVUS analyses (Figure 2B) demonstrated a higher progression rate of fibrous plaque volume in the early group compared with the intermediate ($p < 0.05$) and late ($p < 0.01$) groups.

Treatment differences in plaque volume and composition between sirolimus and CNI

There was significant heterogeneity among the 3 study arms with respect to changes in plaque volume induced by the different treatments ($p = 0.03$ for interaction; Figure 3A). There was significantly less plaque volume progression in favor of sirolimus in the early group (−0.67 mm^3/mm/year, 95% CI −1.10 to −0.25), but not in the intermediate or late groups. After multivariate adjustment for differences in time since heart transplantation, treatment with sirolimus was independently associated with changes in plaque volume in the early group (Table 4). Plaque components analyses (Figure 3B) demonstrated a significant difference of fibrous plaque volume progression in favor of sirolimus in the early group (−0.31 mm^3/mm/year, 95% CI −0.58 to −0.03) ($p = 0.03$ for interaction). On the other hand, there were significant differences in necrotic core ($p = 0.02$ for interaction) and dense calcium volume progression ($p = 0.03$ for interaction) in favor of CNI in the late group (0.15 mm^3/mm/year, 95% CI 0.00 to 0.29; and 0.10 mm^3/mm/year, 95% CI 0.03 to 0.17, respectively).

Table 2 IVUS Findings

	Sirolimus ($N = 61$)	CNI ($N = 85$)	p
IVUS follow-up period (years)	2.9 (2.0,3.6)	2.3 (1.7,3.2)	0.11
Vessel volume index			
Baseline (mm^3/mm)	16.7 ± 4.7	16.8 ± 4.8	0.91
Follow-up (mm^3/mm)	16.7 ± 5.1	17.2 ± 4.3	0.58
Change from baseline (mm^3/mm)	0.0 ± 3.6	0.3 ± 3.0	0.10
Lumen volume index			
Baseline (mm^3/mm)	12.2 ± 4.1	12.1 ± 4.2	0.91
Follow-up (mm^3/mm)	11.2 ± 4.1	11.2 ± 3.6	0.95
Change from baseline (mm^3/mm)	−0.9 ± 3.6[a]	−0.9 ± 3.2[a]	0.98
Plaque volume index			
Baseline (mm^3/mm)	4.6 ± 2.4	4.8 ± 2.5	0.76
Follow-up (mm^3/mm)	5.4 ± 2.8	6.0 ± 3.0	0.25
Change from baseline (mm^3/mm)	0.8 ± 1.5[c]	1.2 ± 1.6[c]	0.004
Plaque components			
Fibrous			
Baseline (mm^3/mm)	0.5 (0.0,1.4)	0.6 (0.0,1.8)	0.37
Follow-up (mm^3/mm)	0.9 (0.2,2.2)	1.1 (0.2,2.4)	0.50
Change from baseline (mm^3/mm)	0.4 ± 1.0[b]	0.4 ± 1.2[c]	0.42
Fibrofatty			
Baseline (mm^3/mm)	0.1 (0.0,0.2)	0.1 (0.0,0.3)	0.30
Follow-up (mm^3/mm)	0.1 (0.0,0.2)	0.1 (0.0,0.3)	0.16
Change from baseline (mm^3/mm)	0.0 ± 0.3	0.0 ± 0.6	0.96
Necrotic core			
Baseline (mm^3/mm)	0.1 (0.0,0.4)	0.1 (0.0,0.4)	0.63
Follow-up (mm^3/mm)	0.3 (0.0,1.1)	0.3 (0.0,0.9)	0.96
Change from baseline (mm^3/mm)	0.3 ± 0.6[c]	0.3 ± 0.6[c]	0.92
Dense calcium			
Baseline (mm^3/mm)	0.0 (0.0,0.1)	0.0 (0.0,0.2)	0.12
Follow-up (mm^3/mm)	0.0 (0.0,0.3)	0.0 (0.0,0.4)	0.89
Change from baseline (mm^3/mm)	0.2 ± 0.4[c]	0.1 ± 0.3[c]	0.25

Data expressed as mean ± SD, or median (interquartile range). CNI, calcineurin inhibitor; IVUS, intravascular ultrasound.
[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$ vs baseline.

Subsequently, changes in plaque volume and components during the 1-year follow-up period after study inclusion were compared between 2 treatment groups among the 3 study arms in a sub-population of 99 patients. A marginally significant interaction ($p = 0.08$ for interaction) was observed favoring sirolimus in the early group (Figure 4A). Plaque components analyses (Figure 4B) showed a significant difference in fibrous plaque volume progression in favor of sirolimus in the early group (−0.78 mm^3/mm/year, 95% CI −1.46 to −0.09) ($p = 0.03$ for interaction).

Discussion

In this study we have demonstrated heterogeneity of CAV development in terms of plaque size and components over time, and suggested significant differential effects on CAV by the timing of switching to sirolimus-based immunosuppression after HTx.

The current serial VH-IVUS revealed an early rapid progression of plaque volume, characterized by the development of fibrous plaque component, followed by late accelerated calcification after HTx. These results extend previous IVUS observations at a single time-point demonstrating that the degree of intimal thickness was most prominent during the first 2 years after HTx[10] and, compositionally, the fibrotic and fibrofatty tissue were predominant early after HTx, whereas dense calcium and necrotic core were observed only in patients with a long transplant evolution time.[11,12] The non-uniformity of chronologic changes in each plaque component after HTx suggests the contribution of different pathophysiologic mechanisms to the development of CAV over time.

Early conversion to sirolimus

The early conversion to sirolimus had beneficial effects on CAV progression, as evidenced by the preventive effects on progression of fibrous plaque components. Previous studies have demonstrated that sirolimus[3] or everolimus[2] in combination with cyclosporine can slow plaque progression in de novo HTx recipients, as well as sirolimus-based

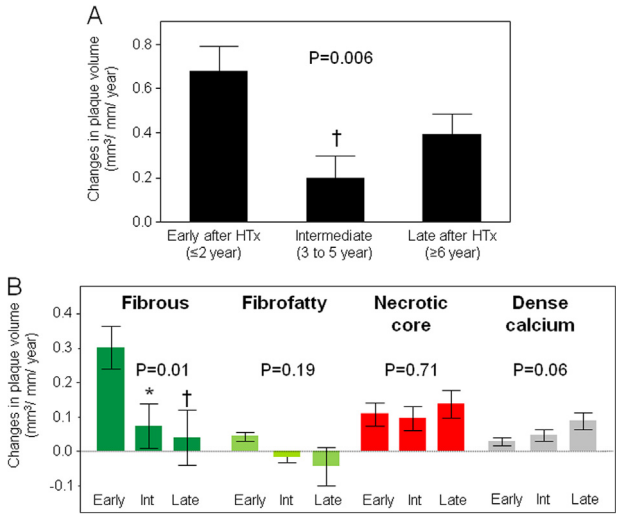

Figure 2 Changes (plaque volume index per year [mm^3/mm/year], mean ± SE) in coronary plaque volume (A) and plaque volume of each component (B) from baseline among 3 groups classified according to the time interval between heart transplantation (HTx) and study inclusion. Early (≤2 years after HTx, $n = 52$), intermediate (Int) (3 to 5 years since HTx, $n = 44$) and late (≥6 years after HTx, $n = 50$) groups are presented. $^{*}p < 0.05$, $^{\dagger}p < 0.01$ vs early group.

immunosuppression with CNI-free regimen in subjects in the early period after HTx.[4–6] Our in vivo observations on plaque composition may provide the potential mechanism to this effect. The results in this study are also consistent with previous experimental findings that sirolimus inhibits activated T-cell proliferation induced by alloantigens, regulates the proliferation and migration of vascular smooth muscle cells, and inhibits extracellular matrix accumulation and fibrotic tissue.[13]

Late conversion to sirolimus

Our observations further suggest that preventive effects on plaque progression may be attenuated when sirolimus is initiated late after HTx. Moreover, late conversion to sirolimus may influence plaque composition, in particular the development of a necrotic core and dense calcium components. Previous studies of PSIs in subjects with a long transplant evolution time have yielded conflicting results. A single-center study demonstrated that sirolimus together with cyclosporine slowed angiographic disease progression,[14] whereas a multicenter study demonstrated that the addition of everolimus to low doses of CNI did not influence plaque volume,[15] but may have shifted plaque composition toward calcified and necrotic plaque components,[16] consistent with our findings.

The mechanisms and clinical significance of an increase in necrotic core and calcium components in subjects with later sirolimus conversion are highly speculative at present. Sirolimus has a systemic effect of destabilizing the metabolic milieu with the development of hyperlipidemia,[17] which may exacerbate CAV. Although earlier studies have demonstrated that hyperlipidemia is associated with CAV, it is unclear whether hyperlipidemia in the face of

Table 3 Sirolimus vs CNI in 3 Study Arms

	Early		Intermediate		Late	
	Sirolimus ($N = 16$)	CNI ($N = 36$)	Sirolimus ($N = 24$)	CNI ($N = 20$)	Sirolimus ($N = 21$)	CNI ($N = 29$)
Recipient age (years)	54 ± 9	54 ± 11	56 ± 11	50 ± 17	56 ± 13	53 ± 16
Male gender [*n* (%)]	9 (56)	28 (78)	17 (71)	13 (65)	17 (81)	24 (83)
Time since heart transplant (years)	1.1 (1.0,1.1)[b]	0.2 (0.2,1.0)	2.2 (2.0,3.0)[b]	3.1 (2.3,5.0)	9.2 (6.5,11.7)	10.9 (8.1,16.6)
Donor age (years)	27 ± 12	33 ± 13	33 ± 14	29 ± 13	26 ± 11	26 ± 12
Previous CMV infection [*n* (%)]	8 (50)	13 (36)	7 (29)	2 (10)	2 (10)	7 (24)
Treatment episodes of rejection [*n* (%)]	2 (13)	6 (17)	4 (17)	1 (5)	4 (19)	6 (21)
Reason for conversion to sirolimus						
CNI-induced nephropathy	4 (25)	–	16 (67)	–	15 (71)	–
CAV	1 (6)	–	1 (5)	–	3 (14)	–
CNI-related side effects	0 (0)	–	2 (9)	–	1 (5)	–
Routine conversion protocol	11 (69)	–	5 (23)	–	2 (10)	–
Comorbidities						
Body mass index (kg/m^2)	27.2 ± 5.9	25.8 ± 4.4	27 ± 5	28 ± 6	28 ± 5	27 ± 6
Diabetes [*n* (%)]	3 (19)	11 (31)	8 (33)	3 (15)	3 (14)	5 (17)
Hypertension [*n* (%)]	12 (75)	24 (67)	19 (79)	13 (65)	17 (81)	21 (72)
Total cholesterol (mg/dl)	228 ± 48	199 ± 48	195 ± 46	188 ± 40	179 ± 31	163 ± 43
Triglyceride (mg/dl)	142 (116,250)	169 (107,210)	148 (107,233)	115 (81,169)	147 (122,194)	125 (78,176)
HDL cholesterol (mg/dl)	54 ± 13	57 ± 22	60 ± 18	53 ± 14	51 ± 18	50 ± 13
LDL cholesterol (mg/dl)	138 ± 34[b]	106 ± 34	101 ± 32	108 ± 33	95 ± 22	86 ± 34
Serum creatinine (mg/dl)	1.1 ± 0.3[a]	1.4 ± 0.3	1.5 ± 0.8	1.4 ± 0.3	1.4 ± 0.3	1.3 ± 0.3

Data are expressed as mean ± SD, median (interquartile range) or number (%). CAV, cardiac allograft vasculopathy; CMV, cytomegalovirus; CNI, calcineurin inhibitor; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a] $p < 0.05$

[b] $p < 0.01$ vs CNI.

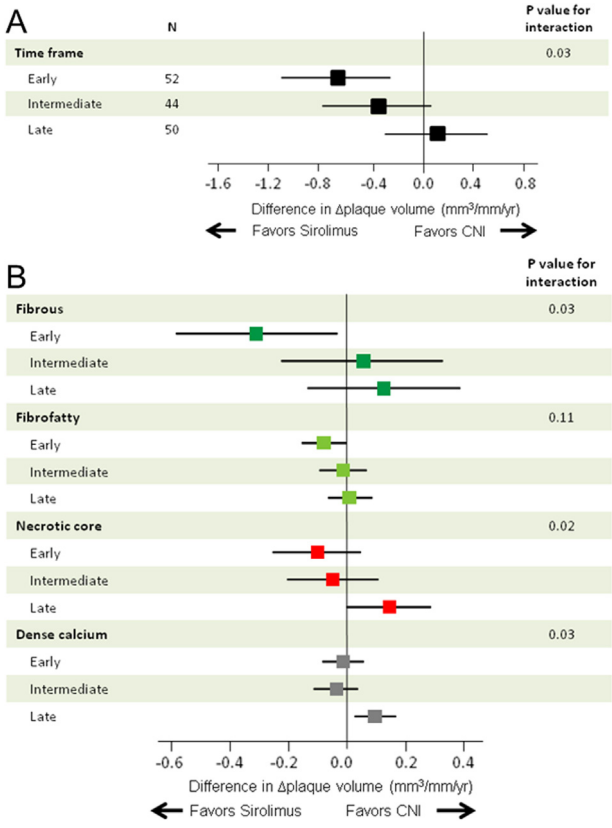

Figure 3 Treatment differences in total plaque volume changes (Δplaque volume) (A) and in Δplaque volume of each plaque component (B) according to the time interval between heart transplantation (HTx) and study inclusion: median follow-up period of 2.8 years. The results are shown as adjusted mean treatment difference (sirolimus minus CNIs) and 95% confidence intervals. Changes in plaque volume were divided by follow-up periods (years) to compensate for the different time interval between 2 IVUS examinations. CNI, calcineurin inhibitor.

sirolimus-based immunosuppression has any negative impact because of the powerful anti-proliferative effects of PSI agents. Clinical observations in native atherosclerosis suggest that culprit lesions responsible for acute coronary syndrome are less calcified compared with those in stable angina, indicating that calcium provides plaque stability.[18] In addition, given a potential misclassification of areas surrounding dense calcium as necrotic core, inherent to VH-IVUS technology,[19] there is a possibility that a parallel increase in necrotic core and dense calcium might be associated with plaque stabilization. In fact, recent VH-IVUS studies of native atherosclerosis showed increases in necrotic core and dense calcium components after statin therapy,[20,21] which is better known for its plaque-stabilizing effects. Given the heterogeneous plaque morphology in CAV,[22] additional research using other imaging techniques is warranted to better characterize plaques in CAV.

Clinical implications

Our study has potential implications for clinical practice. Regarding the natural history of CAV lesions, chronologic heterogeneity of plaque characteristics over time after HTx raises the possibility that optimal therapeutic approaches should consider the time frame after HTx. Furthermore, the initiation of sirolimus early after HTx could offer greater benefits in terms of CAV prevention. Further studies are needed to confirm whether specific plaque types characterized by VH-IVUS are the major determinants of future cardiovascular events in CAV, as was shown in native atherosclerosis.[23]

Limitations

There are several limitations to our investigation. First, our study is limited by its observational design and the small number of patients. Second, there is an inherent selection bias as recipients with extremely progressive CAV, less severe CAV or renal dysfunction may not have follow-up examination. Thus, the present findings may not be extrapolated to these subjects. Third, we analyzed CAV only in the LAD. This could have resulted in an underdiagnosis of CAV, although a previous study demonstrated a good correlation between LAD imaging and the incidence of CAV.[2] Fourth, although comparisons were made using changes in IVUS parameters divided by IVUS follow-up period, this formula does not fully compensate for the different time intervals. This may partially account for variations in the findings between the 2 different IVUS follow-up periods. Fifth, changes in several parameters regarding patient characteristics during the follow-up period may be a source of bias in the IVUS analysis. Sixth, the possibility of residual confounding cannot be ruled out, although the time interval between HTx and study inclusion in the early group was not significant for changes in plaque volume in the multivariate analysis. Finally, as this study did not include subjects undergoing IVUS many years after conversion to sirolimus, any possible long-term effects of sirolimus on CAV were not determined.

In conclusion, sirolimus attenuated plaque progression in recipients with early conversion to sirolimus and induced substantial modification of plaque composition in those with late conversion compared with continued CNI therapy. These observations demonstrate a heterogeneous response

Table 4 Multivariate Predictors for Change in Plaque Volume Index in the Early and Intermediate Groups

	Standardized regression coefficient (*p*-value)			
Variable	Early group		Intermediate group	
Plaque volume index at baseline	0.20	0.20	−0.08	0.87
Treatment with sirolimus	−0.45	0.004	−0.22	0.20
Time since heart transplant	0.20	0.19	0.15	0.39

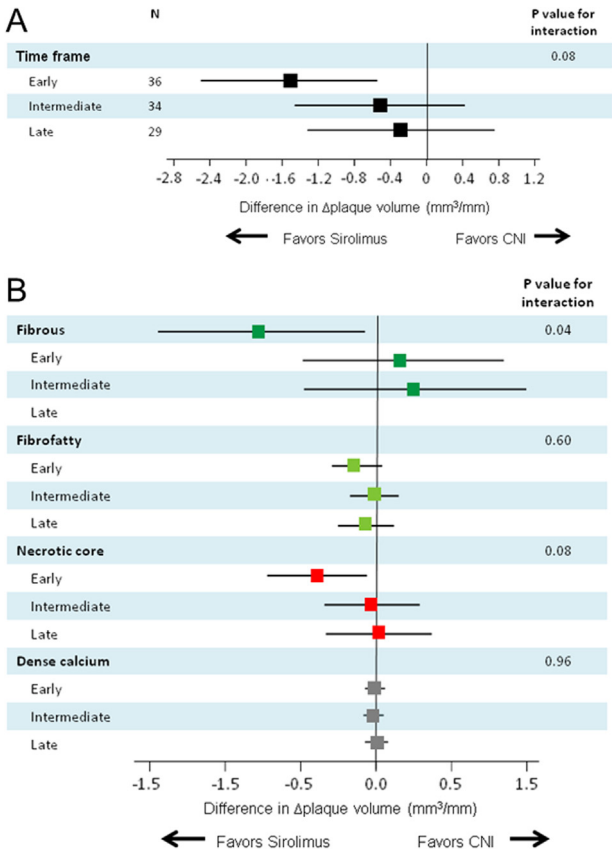

Figure 4 Treatment differences in total plaque volume changes (Δplaque volume) (A) and in Δplaque volume of each plaque component (B) according to the time interval between heart transplantation (HTx) and study inclusion: 1-year follow-up in a sub-population of 99 patients. The results are shown as adjusted mean treatment difference (sirolimus minus CNIs) and 95% confidence intervals. CNI, calcineurin inhibitor.

when switching from CAV to sirolimus, a process that appeared to be dependent on the timing of the switch.

Disclosure statement

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