

Association between the vasa vasorum and the atherosclerotic changes in cardiac allograft vasculopathy: volumetric analysis

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Aims

The current study was designed to test that vasa vasorum (VV) plays a role in the progression of cardiac allograft vasculopathy (CAV) in patients with heart transplantation (HTX).

Methods and results

Intravascular ultrasound (IVUS) and optical coherence tomography (OCT) were performed in the left anterior descending artery in 19 segments of 19 HTX patients (median 2.1 years from HTX). Each segment is composed of both the continuous lesions: (i) CAV area: intimal thickness >0.5 mm with 5 mm length and (ii) VV area: intimal thickness ≤ 0.5 mm with 5 mm length. The per cent VV volume (VV volume/vessel volume $\times 100$, %VV) was evaluated in the VV area with OCT (in CAV area VV cannot be assessed because of limited penetration power of OCT). A year later, the association between the baseline %VV and the change in per cent plaque volume (plaque volume/vessel volume $\times 100$, %PV) was evaluated with IVUS. To a normal distribution, $\Delta\%PV$ (follow-up %PV – initial %PV) was undergone square root transformation. The correlations between the %VV at baseline study and square root- $\Delta\%PV$ were significant both in the CAV area and in the VV area ($r = 0.787$, $P < 0.001$ and $r = 0.701$, $P < 0.001$, respectively). In multivariable analysis, only the %VV was significantly correlated with square root- $\Delta\%PV$ in both areas.

Conclusion

The current study demonstrated a significant association between the VV volume and the progression of plaque volume in both the CAV area and the VV area. Thus, VV may be a potential predictor and possible therapeutic target to attenuate CAV.

Keywords

cardiac allograft vasculopathy • heart transplantation • vasa vasorum

Introduction

Cardiac allograft vasculopathy (CAV) is an accelerated form of intimal hyperplasia of the coronary artery after heart transplantation (HTX) characterized by diffuse progressive luminal narrowing.¹ In some patients, CAV develops as early as 1 year after HTX and is the major cause of long-term morbidity and mortality after HTX.^{2,3}

The vasa vasorum (VV) is a complex plexus of microvessels located in the vessel wall.⁴ Proliferation of the VV has been shown to play an important role in the progression and complications of atherosclerosis and typically precedes the development of intimal

thickening of the coronary artery.^{5,6} CAV shares several common features with atherosclerosis and so we thought that assessment of the adventitial VV may assist in predicting the progression of CAV.^{7,8}

Intravascular ultrasound (IVUS) is a well-established method for evaluating changes in coronary atherosclerotic plaque and has been used to quantify this process in CAV.^{9,10} On IVUS, CAV area could be defined as an intimal thickening of >0.5 mm.¹¹ Optical coherence tomography (OCT) is an emerging tool to evaluate the coronary artery near histological levels including the morphology of adventitial VV.¹² However, measurement of the adventitial VV under CAV area is limited by the penetration power of OCT.

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We hypothesized that the quantitatively assessed VV density, measured adjacent to the CAV area, could represent the VV of the CAV area because of the diffuse disease pattern of the CAV and VV network.^{7,13} This study aimed to evaluate the association between the VV density and the changes in the intimal plaque volume in patients with CAV.

Methods

All HTX recipients with >18 years old were eligible for study unless they had chronic kidney disease \geq Stage IV (glomerular filtration rate <30 mL/min), active infection, active rejection, or were unable to participate in the study. From 14 September 2011 to 9 July 2012, there were 121 consecutive HTX patients who were referred for their annual coronary angiography. Among them, there were 27 patients who underwent both OCT and IVUS of the left anterior descending coronary artery. All of the 27 patients were evaluated follow-up IVUS 1 year later. The following continuous lesions were evaluated for our study: (i) VV area: a lesion consecutive to the proximal or distal part of the CAV area with an intimal thickening \leq 0.5 mm and length >5 mm that would clearly evaluate VV with OCT, and (ii) CAV area: intimal thickness

>0.5 mm with a lesion of >5 mm (Figure 1). For the homogeneity of the study, sample location was limited to the proximal portion of the upper one-third of mid-left anterior descending coronary artery. The scheme of analysis is described in Figure 2. The study protocols were approved by the institutional review board of the Mayo Clinic. All patients gave their written informed consents to participate in this study.

IVUS image acquisition and analysis

The IVUS examination was performed as previously described.¹⁴ In brief, after intracoronary administration of 100–200 mg nitroglycerin, a 20 MHz IVUS catheter (Eagle Eye Gold, Volcano Corporation, Rancho Cordova, CA, USA) was advanced into the middle to distal left anterior descending coronary artery, and automatic pullback at 0.5 mm/s was done. Offline volumetric reconstruction analyses of IVUS were performed by one independent experienced examiner, who is blinded to the clinical data and the OCT findings, using the Volcano Image Analysis software V3.1 (Volcano Corporation). Quantitative volumetric analysis was performed in the 5-mm segment of both the CAV area and the VV area. The average vessel volume (mm^3) was calculated by average vessel area (mm^2) \times 5-mm lesion length. The per cent plaque volume (%PV) was determined by (plaque volume/average vessel volume) \times 100. The difference between the follow-up %PV and the initial %PV was expressed as

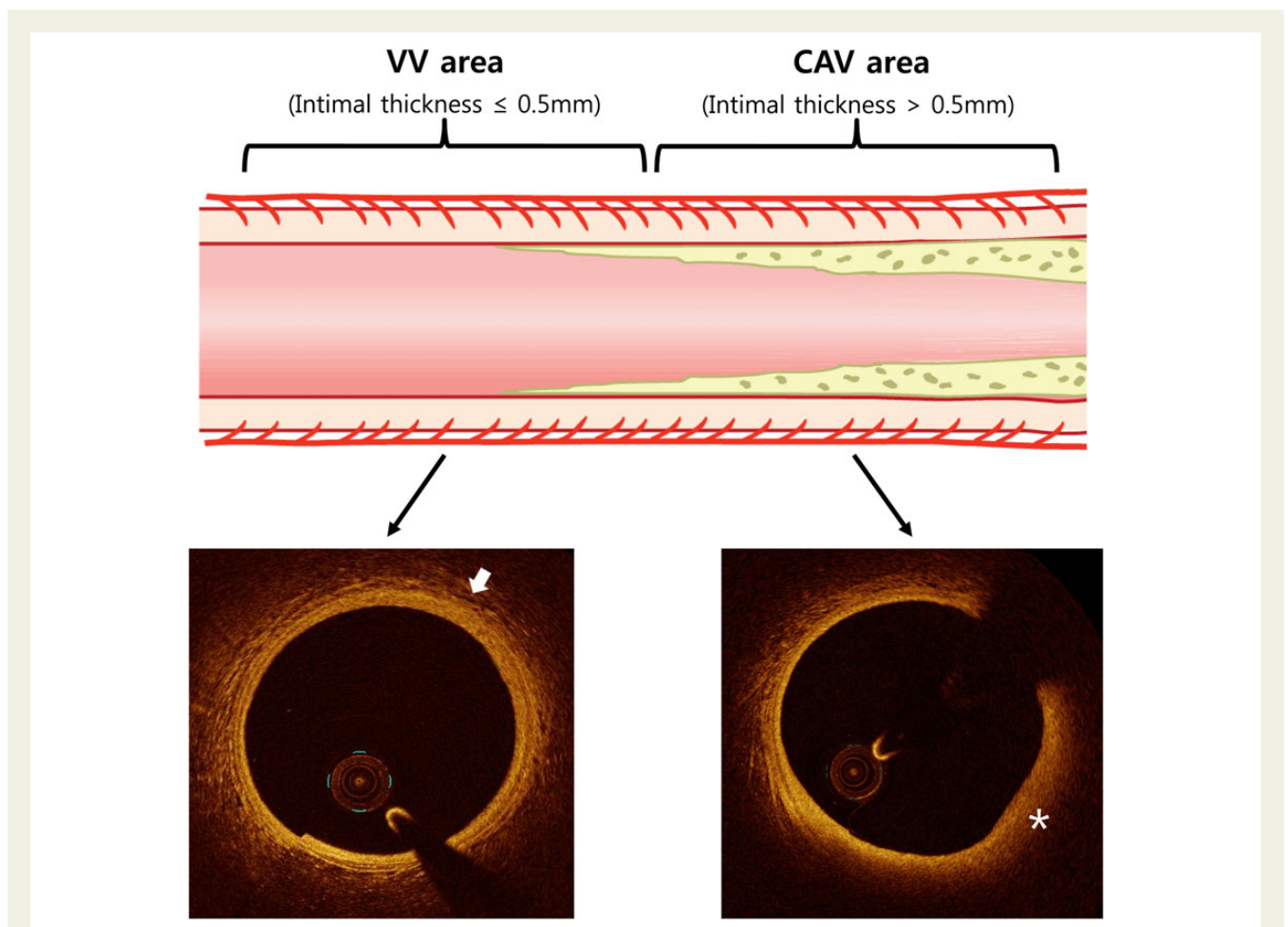


Figure 1 This illustration shows the study segment of the study. In the VV area, which is adjacent to the CAV area, the VV can be clearly evaluated with OCT (arrow). In the CAV area, the evaluation of the VV in plaque area (asterisk) is difficult because of the limited penetration power of the OCT. CAV, coronary allograft vasculopathy; VV, vasa vasorum.

the delta per cent plaque volume ($\Delta\%PV$). A total of 15 segments of IVUS were analysed by two independent examiners to evaluate inter-observer and intra-observer variabilities on plaque volume. Intra-observer agreement was calculated 10 days after the first study. The Lin's concordance correlation coefficient values for the inter-observer and intra-observer agreement were 0.947 and 0.971, respectively.

OCT image acquisition and analysis

Acquisition and analysis of OCT images were performed also as previously described.¹⁵ For the acquisition of OCT images, the C7-XR OCT intravascular imaging system (St Jude Medical, St Paul, MN, USA) was

used. The imaging catheter (Dragonfly, St Jude Medical) was advanced into the mid-to-distal segment of the left anterior descending coronary artery, and automatic pullback of 50 mm at a speed of 20 mm/s (100 frames/s) was initiated in concordance with the blood clearance through the infusion of the radiocontrast media. All OCT images were digitally stored and analysed offline using LightLab imaging (St Jude Medical). We excluded segments that contained poor images to observe the VV in the adventitia of coronary artery because of the incomplete blood removal or plaque thickness >0.5 mm, because the light signal of the OCT could be attenuated in such segments. We also excluded segments with major branches that occupied >90 degrees of vessel wall, which obscure VV in the vessel adventitia.

Volumetric analysis of the VV and its matched vessel in the VV area was performed using the ANALYZE software 11.0 (Biomedical Imaging Resource, Rochester, MN, USA) which was demonstrated as the useful modality of 3D volumetric generation and analysis of OCT images as previously described.¹⁶ First, the data of the OCT images were converted in to the DICOM file. On the ANALYZE program, the DICOM file of the OCT images was loaded as red channel data with an 8-bit matrix of $20 \times 20 \times 200$ μm cubic voxels. The 5-mm length of the VV area was traced in every slice of the cross-sectional OCT image by a distance of 200 μm . Adventitial VV was defined as signal-voiding tubular structures with major diameters of 50–300 μm , observed in at least two consecutive slices, and located within 1 mm from the lumen-intima border.¹⁶ After determining and tracing each VV in all of separate slice (Figure 3), we set the range of slice number that would be analysed. The analysis of VV volume rendering process was done by the voxel number \times cubic voxel size of VVs according to the continuous frame. The vessel volume was defined as the reconstructed volume surrounded by the boundaries between the media and the adventitia of coronary artery. The VV volume fraction (%VV) was expressed as (VV volume/vessel volume) \times 100. Image analysis was performed by one examiner who is well-trained to analyse OCT images and blinded to the clinical data and IVUS findings. To evaluate inter-observer and intra-observer variabilities on volumetric analysis of VV with OCT, a total of 100 image slices were analysed by two independent assessors on VV volume. Intra-observer agreement was calculated 14 days after the first study. The Lin's concordance correlation coefficient values for the inter-observer and intra-observer agreement were 0.905 and 0.919, respectively.

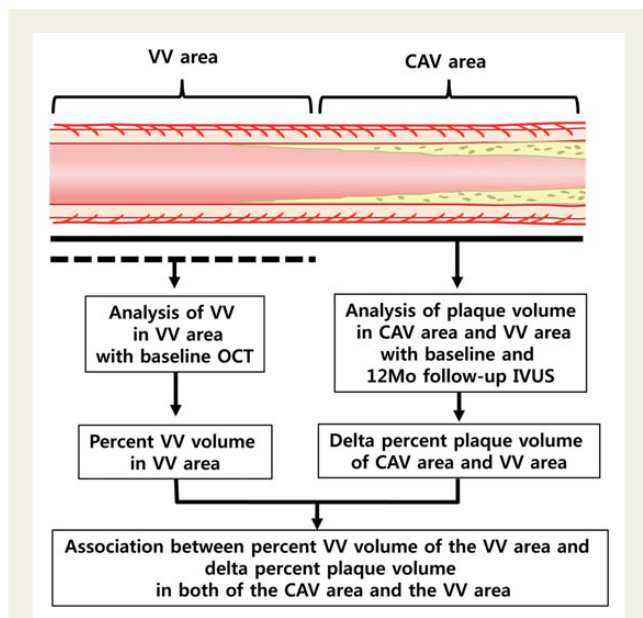


Figure 2 This is the scheme of analysis of the study. CAV, coronary allograft vasculopathy; OCT, optical coherence tomography; IVUS, intravascular ultrasound; VV, vasa vasorum.

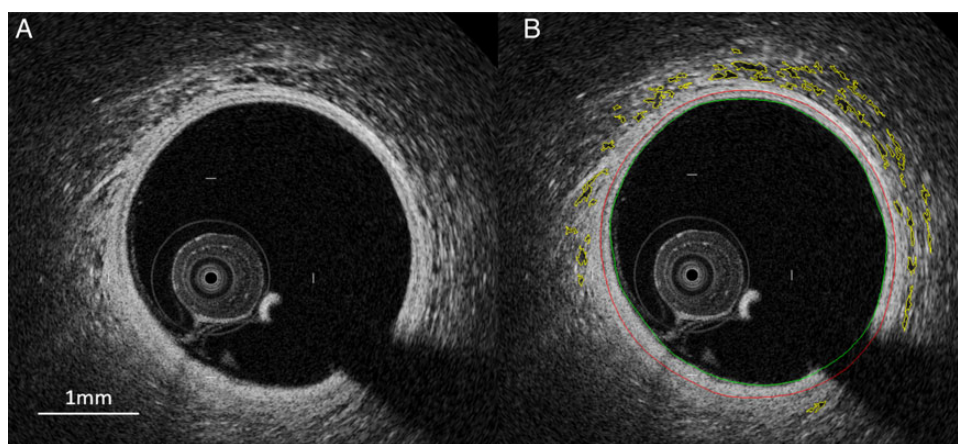


Figure 3 After storage with the DICOM file, OCT image converted to red channel image data (A). The same image with boundaries of the VV is shown in (B). Yellow lines indicate the areas of VV.

To match the study lesion location between OCT and IVUS, anatomical landmarks such as side branches and calcium were used. The coronary angiography was also used to get the information of the distribution and location of the side branches. The distance from the anatomical landmark to each study segment point was determined using the longitudinal reconstructed OCT and IVUS image sets to ensure that distance measurements were the same between the both methods. All matching between OCT and IVUS images was done by an independent assessor who is blinded to the volumetric analysis of IVUS and OCT. Another independent observer confirmed that the OCT, baseline IVUS, and follow-up IVUS locations were same for all study segments.

Statistical analysis

Continuous variables are denoted as the mean \pm standard deviation or median and inter-quartile range (25 and 75% quartiles) as appropriate. Discrete variables are presented as frequency (percentage). Comparisons between index and follow-up measures were tested with Wilcoxon signed rank test. Comparisons between independent groups were conducted with Mann–Whitney *U* test. Correlation between variables was evaluated with Pearson's or Spearman's rank correlation as appropriate. As the $\Delta\%PV$ (follow-up $\%PV$ – initial $\%PV$) in the CAV area and the VV area showed a non-normal distribution, a square root transformation was applied to the $\Delta\%PV$ for normal distribution ($W = 0.975$, $P = 0.870$). Multivariable linear regression analysis was performed to study the independent correlates of the change in the $\%PV$. Potential collinearity was considered acceptable, and the regression model was stable at a variance inflation factor of <4 . All statistical tests were two-sided, and a *P*-value of <0.05 was considered statistically significant. Statistical analysis was performed using JMP 10.0 (SAS Institute, Cary, NC, USA).

Results

Among the 27 patients, 3 patients had poor OCT image and 5 patients did not have a suitable lesion segment for the study because of the diffuse CAV lesions. Finally, 25 segments in 19 patients were registered for analysis. Because six of them also had a study lesion at the lower mid or distal left anterior descending coronary artery, they were excluded from analysis, leaving 19 samples.

The baseline clinical characteristics are shown in Table 1. The follow-up study was conducted after 12.5 ± 1.2 months. OCT analysis in VV area showed that the vessel volume was 60.5 ± 18.1 mm³ and VV volume was 2.1 ± 0.6 mm³, and thus, the $\%VV$ was $3.6 \pm 0.9\%$. The mean total VV count was 835 ± 266 . The initial and follow-up volumetric analysis of the grey-scale IVUS is shown in Table 2. The average lumen area, vessel area, and vessel volume were not different between the initial and the follow-up IVUS studies in either the CAV area or the VV area. However, compared with the initial study, the follow-up plaque volume and the $\%PV$ were significantly increased both in the CAV area and in the VV area. Between the $\%VV$ and the initial $\%PV$, there was significant correlation of the CAV area and marginal correlation of the VV area ($r = 0.597$, $P = 0.007$ and $r = 0.416$, $P = 0.077$, respectively). Table 3 shows the correlation between the square root- $\Delta\%PV$ and the $\%VV$ and other risk factors of CAV. In both the CAV area and the VV area, $\%VV$ was significantly correlated with the square root- $\Delta\%PV$ ($r = 0.787$, $P < 0.001$ and $r = 0.701$, $P < 0.001$, respectively, Figure 4). Among the other risk factors, only years after HTX was significantly correlated with the

Table 1 Baseline clinical characteristics

Variables	Values (n = 19)
Age, median (Q1, Q3)	54 (46, 65)
Male sex, n (%)	12 (63.2)
Current smoking, n (%)	0 (0.0)
Diabetes, n (%)	6 (31.6)
Hypertension, n (%)	12 (63.2)
Dyslipidaemia, n (%)	11 (57.9)
Renal insufficiency, n (%)	11 (57.9)
Body mass index, kg/m ²	27.8 \pm 4.8
Systolic blood pressure, mmHg	109 \pm 9
Diastolic blood pressure, mmHg	70 \pm 8
Heart rate, bpm	80 \pm 11
Reason for HTX	
Ischaemic CMP, n (%)	3 (15.8)
Non-ischaemic CMP, n (%)	16 (84.2)
Years after HTX, median (Q1, Q3)	2.1 (1.0, 8.0)
Donor age, median (Q1, Q3)	30 (21, 41)
Cold ischaemic time, min	191 \pm 34
CMV infection, n (%)	6 (31.6)
LVEF (%)	65.3 \pm 4.5
ISHLT grade	
CAV0, n (%)	7 (36.8)
CAV1, n (%)	10 (52.6)
CAV2, n (%)	1 (5.2)
CAV3, n (%)	1 (5.2)
Laboratory data	
Total cholesterol (mg/dL)	200 \pm 42
Triglyceride (mg/dL)	186 \pm 106
HDL (mg/dL)	57 \pm 17
LDL (mg/dL)	107 \pm 27
Creatinine (mg/dL)	1.2 \pm 0.3
Fasting blood sugar (mg/dL)	112.8 \pm 61.4
Medication	
Aspirin, n (%)	3 (15.8)
Statin, n (%)	18 (94.7)
Steroid, n (%)	10 (47.4)
Azathioprine, n (%)	3 (15.8)
Cyclosporin, n (%)	3 (15.8)
Mycophenolate mofetil, n (%)	13 (68.4)
Sirolimus, n (%)	13 (68.4)
Tacrolimus, n (%)	3 (15.8)

BMI, body mass index; CAV, cardiac allograft vasculopathy; CMP, cardiomyopathy; CMV, cytomegalovirus; HDL, high-density lipoprotein; HTX, heart transplantation; ISHLT, International Society for Heart and Lung Transplantation; LDL, low-density lipoprotein.

square root- $\Delta\%PV$ in the CAV area ($r = 0.678$, $P = 0.001$). In the *post hoc* analysis, which included six segments of the lower two-thirds, the result also showed a significant correlation between the $\%VV$ and the square root- $\Delta\%PV$ both in the CAV area and in the VV area ($r < 0.776$, $P < 0.001$ and $r < 0.756$, $P < 0.001$, respectively).

Table 2 Grey-scale data of the intravascular ultrasound

	Initial study (n = 19)	FU study (n = 19)	P-value ^a
CAV area (5 mm)			
Average lumen area (mm ²)	10.3 ± 2.9	9.4 ± 3.6	0.133
Average vessel area (mm ²)	13.3 ± 3.1	13.4 ± 3.9	0.852
Average vessel volume (mm ³)	66.7 ± 15.1	67.1 ± 19.5	0.852
Plaque volume (mm ³)	15.4 ± 3.9	20.8 ± 6.5	<0.001
Per cent plaque volume (%)	23.9 ± 7.1	32.8 ± 11.2	<0.001
Vasa vasorum area (5 mm)			
Average lumen area (mm ²)	9.9 ± 2.5	9.2 ± 3.4	0.215
Average vessel area (mm ²)	11.9 ± 2.7	11.8 ± 3.6	0.859
Average vessel volume (mm ³)	59.5 ± 13.5	59.0 ± 18.1	0.859
Plaque volume (mm ³)	10.1 ± 2.8	12.8 ± 3.4	<0.001
Per cent plaque volume (%) ^b	17.4 ± 5.0	23.2 ± 7.6	<0.001

CAV, cardiac allograft vasculopathy; FU, follow-up.

^aAll P-values are from the Wilcoxon signed rank test.

^b(Plaque volume/average vessel volume) × 100.

Table 3 Relations of square root-Δ%PV with the clinical parameters for cardiac allograft vasculopathy

	Correlation coefficient	P-value ^a
CAV area (5 mm)		
%VV	0.787	<0.001
Patient age	-0.086	0.726
Donor age	0.199	0.413
Years after HTX	0.678	0.001
Body mass index	0.075	0.759
Ischaemic time	0.142	0.562
Vasa vasorum area (5 mm)		
%VV	0.701	<0.001
Patient age	0.044	0.855
Donor age	0.277	0.236
Years after HTX	0.242	0.304
Body mass index	0.300	0.199
Ischaemic time	0.381	0.097

CAV, cardiac allograft vasculopathy; HTX, heart transplantation; %VV, per cent vasa vasorum volume; Δ%PV, delta per cent plaque volume.

^aAll P-values are from the Spearman's correlation except P-value of %VV from the Pearson's correlation.

Although some of the univariate results were not statistically significant, multivariable analysis was performed using all the variables, because known clinical relevance as risk factors for progression of CAV. The multivariable linear regression showed that only the %VV and the square root-Δ%PV were independently correlated in both the CAV area and the VV area (Table 4). Table 5 shows progression of %PV within risk factor subgroups (defined by sex, years after HTX, donor age, and other possible risk factors for CAV and the progression of atherosclerosis). The result showed that the group

divided by years after HTX >2 years (median year in this study) significantly increased in terms of the Δ%PV compared with the group <2 years after HTX (12.8 ± 6.3 vs. 3.6 ± 2.3%, P = 0.001).

Discussion

In this study, we showed that VV volume is one of the major determinant factors of the intimal plaque progression in patients with CAV. To the best of our knowledge, this report is the first to demonstrate the progression of plaque volume associated with VV volume using volumetric analysis.

CAV is an atheromatous disease and shares common features with atherosclerosis. In the early phase, both diseases are characterized by endothelial dysfunction followed by increased cell adhesion, leucocyte infiltration, intracellular lipids accumulation, and smooth muscle proliferation.^{7,8,17} Compared with native atherosclerosis, CAV has a tendency to undergo rapid and accelerated development.⁷ The prevalence of CAV is high even in the first year after HTX.² Moreover, the progression of intimal thickness in the first year after HTX has been reported to be a significant predictor of future cardiac events.³ Therefore, prevention and early detection of CAV remain an important goal to improve the prognosis of the patients with HTX.

Previous studies have shown that neovascularization of the VV plays a significant role in both early and advanced phases of atherosclerosis.^{6,18} The close proximity of the VV to the intima makes it susceptible to infiltration of inflammatory cells and expression of the adhesion molecule. This susceptibility suggests that the VV serves as a conduit for the entry of pro-inflammatory and pro-atherosclerotic components into the wall of the coronary artery.¹⁹ Neovascularization of the VV appears to be a key feature even prior to the development of atherosclerosis. In a swine model, adventitial neovascularization of the VV is present early after a hypercholesterolaemic diet, preceding endothelial dysfunction and development of atherosclerosis.⁶ Previous human autopsy studies

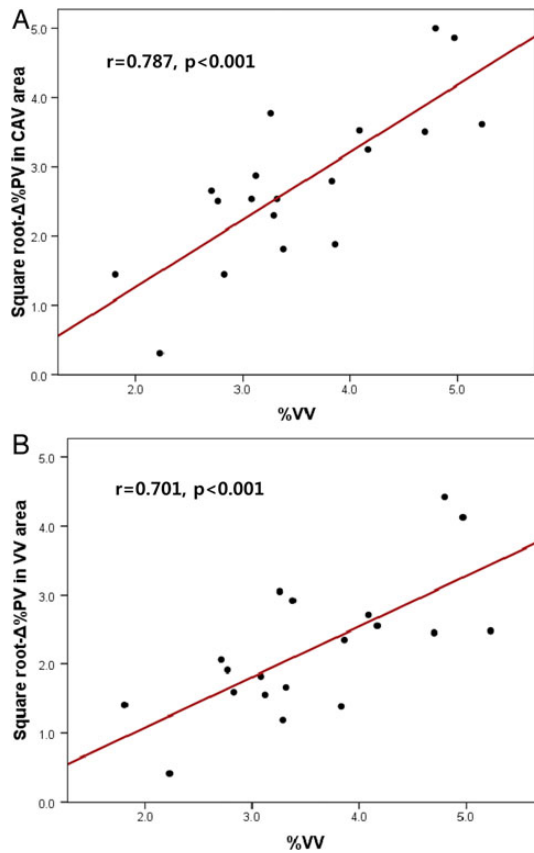


Figure 4 In both the CAV area (A) and the VV area (B), the %VV is significantly correlated with the square root-Δ%PV. Square root-Δ%PV, square root delta per cent plaque volume; %VV, per cent vasa vasorum volume; CAV, cardiac allograft vasculopathy; VV, vasa vasorum.

using microcomputed tomography have also shown that the VV density correlates well with the amount of advanced atherosclerotic plaque. Moreover, the VV density of the non-stenotic segment was higher than the VV density of the normal segment.⁵ In the present study, the %VV of the VV area significantly correlated with the progression of the intimal plaque volume both in the CAV area and in the VV area. These observations suggest that the measurement of the VV volume may enable the prediction of the initiation and progression of CAV in patients following HTX. Our observations suggest that controlling the neovascularization of the VV may provide a potential therapeutic target, a concept that is supported by previous observations. In a swine model of atherosclerosis induced by a high cholesterol diet, anti-angiogenic treatment targeting neovascularization of the VV leads to preservation of the VV density, inhibition of the vascular endothelial growth factor expression, and atherosclerosis progression.^{20,21}

Compared with coronary angiography, IVUS provides not only accurate information on luminal stenosis but also changes in vessel size. In patients with HTX, a 5-year serial IVUS study revealed that changes in the external elastic membrane area showed a biphasic response that involved expansion in early phase and constriction in the late period.⁹ In this study, the *post hoc* analysis showed that the follow-up vessel area showed a decreasing trend compared with the initial vessel area (9.9 ± 2.8 vs. 11.1 ± 2.4 mm², $P = 0.125$) in patients with >5 years after HTX, consistent with the previous report. There was no significant correlation between %VV and the delta average vessel volume in the CAV area and in the VV area ($r = 0.001$, $P = 0.983$ and $r = -0.209$, $P = 0.391$, respectively). In IVUS system, specific histologic plaque components can be identified with virtual histology analysis.¹⁰ In this study, changes of the fibrotic and fibro-fatty components of the CAV area were significantly correlated with %VV in terms of absolute volume and per cent absolute volume (see Supplementary data online, *Table S1* and *S2*). Therefore, the quantitative histologic characteristics of

Table 4 Results of the multivariable linear regression to assess the independent correlates of the square root-Δ%PV

	SD	SC-β	95% CI for SC-β	P-value	VIF
CAV area (Model F ratio = 6.504, P = 0.003)					
%VV	0.93	0.657	0.319 to 1.194	0.003	1.554
Years after HTX	3.57	0.332	-0.071 to 0.843	0.092	1.679
Patient age	12.20	0.086	-0.281 to 0.488	0.585	1.202
Donor age	10.72	0.212	-0.151 to 0.654	0.211	1.314
Body mass index	4.51	-0.033	-0.469 to 0.392	0.849	1.490
Ischaemic time	33.61	0.141	-0.202 to 0.538	0.362	1.125
Vasa vasorum area (Model F ratio = 3.812, P = 0.021)					
%VV	0.93	0.597	0.160 to 0.969	0.010	1.406
Years after HTX	3.57	0.241	-0.214 to 0.693	0.275	1.607
Patient age	12.20	0.074	-0.317 to 0.464	0.685	1.156
Donor age	10.72	0.224	-0.182 to 0.622	0.254	1.264
Body mass index	4.51	0.077	-0.347 to 0.541	0.706	1.412
Ischaemic time	33.61	0.182	-0.202 to 0.571	0.316	1.092

CAV, cardiac allograft vasculopathy; CI, confidence interval; HTX, heart transplantation; %VV, per cent vasa vasorum volume; SD, sample standard deviation; Δ%PV, delta per cent plaque volume; SC-β, standardized coefficients β; VIF, variance inflation factor.

Table 5 Change in the %PV in the cardiac allograft vasculopathy area according to clinical factors

	Initial %PV	Follow-up %PV	Δ %PV ^a	P-value of within factor ^b	P-value of between factor ^c
Sex					0.566
Male (n = 12)	22.4 ± 5.3	30.6 ± 10.9	8.2 ± 7.2	0.016	
Female (n = 7)	26.3 ± 9.4	36.5 ± 11.4	10.1 ± 6.3	<0.001	
Years after HTX					0.001
≤2 years (n = 8)	20.4 ± 3.3	24.0 ± 4.9	3.6 ± 2.2	0.008	
>2 years (n = 11)	26.4 ± 8.2	39.1 ± 10.0	12.8 ± 6.3	0.001	
Donor age					0.186
≤30 years (n = 10)	22.2 ± 5.43	29.2 ± 9.6	7.0 ± 6.7	0.002	
>30 years (n = 9)	25.7 ± 8.6	36.81 ± 1.9	11.1 ± 6.5	0.004	
Diabetes					0.550
Yes (n = 6)	20.3 ± 3.8	27.8 ± 3.7	7.5 ± 4.3	0.031	
No (n = 13)	25.5 ± 7.8	35.1 ± 12.7	9.6 ± 7.7	<0.001	
Dyslipidaemia					0.299
Yes (n = 11)	25.4 ± 7.4	35.8 ± 11.6	10.3 ± 7.6	0.001	
No (n = 8)	21.7 ± 6.4	28.7 ± 9.5	7.0 ± 5.2	0.008	
Renal insufficiency					0.547
Yes (n = 11)	24.7 ± 8.0	34.5 ± 12.0	9.7 ± 6.4	0.001	
No (n = 8)	22.6 ± 5.9	30.4 ± 10.2	7.8 ± 4.5	0.002	
CMV infection					0.511
Yes (n = 6)	22.9 ± 3.2	29.8 ± 6.8	6.9 ± 4.1	0.031	
No (n = 13)	24.3 ± 8.4	34.1 ± 12.6	9.9 ± 7.6	<0.001	
ISHLT rejection grade ≥2					0.118
Yes (n = 7)	23.7 ± 6.7	35.2 ± 11.2	11.5 ± 7.1	0.016	
No (n = 12)	23.9 ± 7.6	31.4 ± 11.3	7.4 ± 6.4	<0.001	
Underlying disease					0.336
Ischaemic CMP (n = 3)	21.1 ± 3.3	26.5 ± 7.4	5.4 ± 4.5	0.250	
Non-ischaemic CMP (n = 16)	24.4 ± 7.6	33.9 ± 11.5	9.6 ± 7.0	<0.001	
Maintenance regimen					0.895
Sirolimus (n = 13)	23.2 ± 5.1	32.5 ± 10.0	9.3 ± 7.4	<0.001	
Calcineurin inhibitors (n = 6)	25.3 ± 10.8	33.5 ± 14.3	8.2 ± 5.7	0.031	

CMV, cytomegalovirus; CMP, cardiomyopathy; HTX, heart transplantation; ISHLT, International Society for Heart and Lung Transplantation; %PV, per cent plaque volume.

^aDifference between follow-up %PV and initial %PV.

^bP-values are from the Wilcoxon signed rank test.

^cP-values are from comparing the Δ %PV between factors with the Mann–Whitney *U* test.

plaque associated with the control VV could be another potential target in the treatment of CAV.

OCT is a high-resolution (10 μ m), light-based, and *in vivo* imaging modality capable of detecting near-microscopic histological findings. A recent study showed that human adventitial VV could be evaluated with OCT *in vivo*.¹² Moreover, we demonstrated the quantitative measurement of the adventitial VV volume with OCT and the significant correlation between the %VV and the %PV among patients in the early period after HTX.¹⁶ In our previous study, we used the ANALYZE software, which could calculate the VV volume from conventional intravascular OCT. Compared with the micro-CT, which is an established tool for the imaging of VV *in vitro*, there were significant correlations between the count and volume of the VV between both modalities. In addition, we analysed OCT imaging with same software in eight recipients for the evaluation of CAV. There was a significant positive correlation between the VV volume and plaque volume. However, our previous study was

a cross-sectional study. Going one step further, we demonstrated that significant correlation between the VV volume and the progression of CAV in this study.

Analyses of risk factors in patients with CAV have identified both immune-mediated injury and non-immunologic factors.¹ Immune-mediated injury includes HLA mismatch, the episode and grade of rejection, etc. Non-immunologic risk factors include hyperlipidaemia, donor's age, sex, diabetes mellitus, renal insufficiency, cytomegalovirus infection, and ischaemic time. In addition, recent studies have shown that sirolimus-based maintenance therapy attenuated plaque progression compared with calcineurin inhibitors after HTX.²² In this study, we compared the group of patients according to their CAV risk factors. The analysis showed that the %PV significantly progressed more in the group with patients >2 years after HTX than with those <2 years after HTX. Thus, although conventional risk factors of CAV and cardiovascular risk factors were not significantly affected in this study, duration of

exposure to multiple risk factors, such as insulin resistance, renal dysfunction, and various effects, could play a role in the ongoing immunosuppressive therapy.

Limitations

This study had several potential limitations. First, the lengths of the VV area and the CAV area evaluated for plaque progression were relatively short, and the study analysis was limited to the proximal and the upper one-third of mid-left anterior descending coronary artery. Thus, generalizing our result to all the territories of the coronary artery after HTX is difficult. Second, several segments were excluded because of low image quality due to incomplete flushing with the radiocontrast media. This exclusion could introduce a selection bias. Moreover, on account of the limited penetration power of the OCT, we evaluated VV within 1 mm from the lumen. Therefore, the VV could have been underestimated in the study. Third, even though all matching between OCT and IVUS images were done according to the anatomical landmarks, considering the inherent differences in both techniques, this general matching process will be somewhat inaccurate to provide the same exact location. Fourth, in terms of the number of patients, there was a small sample size, and the various periods after HTX were mixed. It remains unclear whether the results could be confirmed in a larger patient sample with specific period. Fifth, the mean follow-up period was only 12 months. Therefore, further studies with a specific period and a larger number of patients with long-term follow-up are needed to confirm this observation.

Conclusions

The study demonstrated the strong correlation between the VV volume and the progression of the plaque volume in both the CAV area and the VV area. In a multivariable model, the VV volume was the only factor significantly associated with plaque progression. This result supports the association between the extent of VV and the development of CAV. Therefore, VV could be a potential therapeutic target to prevent CAV after HTX.

Supplementary data

Supplementary data are available at *European Heart Journal—Cardiovascular Imaging* online.

Conflict of interest: none declared.

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