

RESEARCH ARTICLE

Progression of cardiac allograft vasculopathy assessed by serial three-vessel quantitative coronary angiography

Christian Zanchin¹, Kyohei Yamaji¹, Carolin Rogge¹, Dorothea Lesche², Thomas Zanchin¹, Yasushi Ueki¹, Stephan Windecker¹, Paul Mohacsi¹, Lorenz Räber¹, Vilborg Sigurdardottir^{1*}

1 Department of Cardiology, Bern University Hospital, Bern, Switzerland, **2** Institute of Clinical Chemistry, Bern University Hospital, Bern, Switzerland

* vilborgs@gmail.com



Abstract

Background

The purpose of the present study was to assess the short- and long-term progression of cardiac allograft vasculopathy (CAV) using serial 3-vessel quantitative coronary angiography (QCA).

Methods

CAV progression was assessed using serial 3-vessel QCA analysis at baseline, 1-year and long-term angiographic follow-up (8.5±3.7 years) after heart transplantation. The change in minimal lumen diameter (MLD) and percent diameter stenosis (%DS) was serially assessed within matched segments. Patients were graded according to the ISHLT-CAV classification and grouped as ISHLT-CAV₀ and ISHLT-CAV₁₋₃. The primary endpoint was mean change in MLD and %DS.

Results

A total of 41 patients and 520 matched segments were available for serial 3-vessel QCA. Overall, MLD decreased non-significantly from baseline to 1-year follow-up and significantly from 1-year to the long-term angiographic follow-up (Δ -0.08mm/year [95%CI -0.11 to -0.05], $P < 0.001$). %DS increased significantly from baseline to 1-year (Δ +0.96%/year [95%CI 0.04 to 1.88], $P = 0.041$) and from 1-year to long-term angiographic follow-up (Δ +0.61%/year [95%CI 0.33 to 0.88], $P < 0.001$). ISHLT-CAV₁₋₃ at 1 year and at long-term angiographic follow-up was observed in 22% and 61%, respectively. Between baseline and long-term angiographic follow-up, a significant reduction in MLD was observed within both groups without a significant difference in the reduction between the two groups (ISHLT-CAV₀: median -0.49mm [IQR -0.54 to -0.43] vs. ISHLT-CAV₁₋₃: median -0.40mm [IQR -0.44 to -0.35], $P = 0.4$).

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Conclusion

The current data suggest that QCA can't predict CAV beyond 1 year, but, QCA affirmed that CAV progresses to a similar extent in patients who do not develop visual CAV during long-term follow-up.

Background

Cardiac allograft vasculopathy (CAV) has become one of the most important cause of long-term mortality after heart transplantation (HTx) [1]. CAV is characterized by a concentric and diffuse proliferation of the coronary arterial intima, resulting in thickening and progressive luminal narrowing [2]. Because the modification in immunosuppressive therapy may delay or even cause CAV regression, the early detection of CAV is of high clinical interest [3–7].

The current guidelines recommend annual or biannual coronary angiography for detection and surveillance of CAV [5]. CAV is classified on a visual assessment of coronary angiographic findings and graft function according to the International Society for Heart and Lung Transplantation (ISHLT) classification [5]. When using the ISHLT-classification, the incidence of CAV is approximately 8% at 1 year, 30% at 5 years, and 50% at 10 years [1]. However, concern remains in the ability of coronary angiography to detect accurately the early stages and progression of CAV [8]. Intravascular ultrasound (IVUS) studies have found a coronary intimal thickening in up to 80% of patients already during the first year after HTx without angiographic signs of CAV and angiographically silent progression of CAV predicts long-term morbidity and mortality after cardiac transplantation [9,10].

Quantitative coronary angiography (QCA) has been used for many years in clinical research to assess the luminal diameter of arteries. It can readily survey the entire coronary vascular system on the coronary angiographies. While QCA makes quantification of coronary atherosclerosis progression possible [11,12], no studies with serial 3-vessel QCA have been conducted to determine CAV progression. Therefore, the aim of this study was to assess CAV progression by using serial 3-vessel QCA at baseline, at 1 year, and at long-term angiographic follow-up after HTx.

Methods

Study population

This is a retrospective observational cohort study including 83 HTx recipients who underwent heart transplantation between January 1994 and October 2015 at the University Hospital of Bern. Follow-up angiography 1 year after HTx was available in 77 patients and was used to assess CAV risk factors. For the purpose of the serial 3-vessel QCA analyses, we excluded patients without baseline angiography, 1 year follow-up angiography, or long-term follow-up angiography (n = 36). Finally, we enrolled 41 patients in the serial 3-vessel QCA analyses set. A detailed patient flow is shown in Fig 1.

The study complied with the Declaration of Helsinki and the study was approved by the Cantonal Ethics Committee of Bern (CEC 186/14). All patients provided written informed consent. None of the transplant donors were from a vulnerable population and all donors or next of kin provided written informed consent that was freely given.

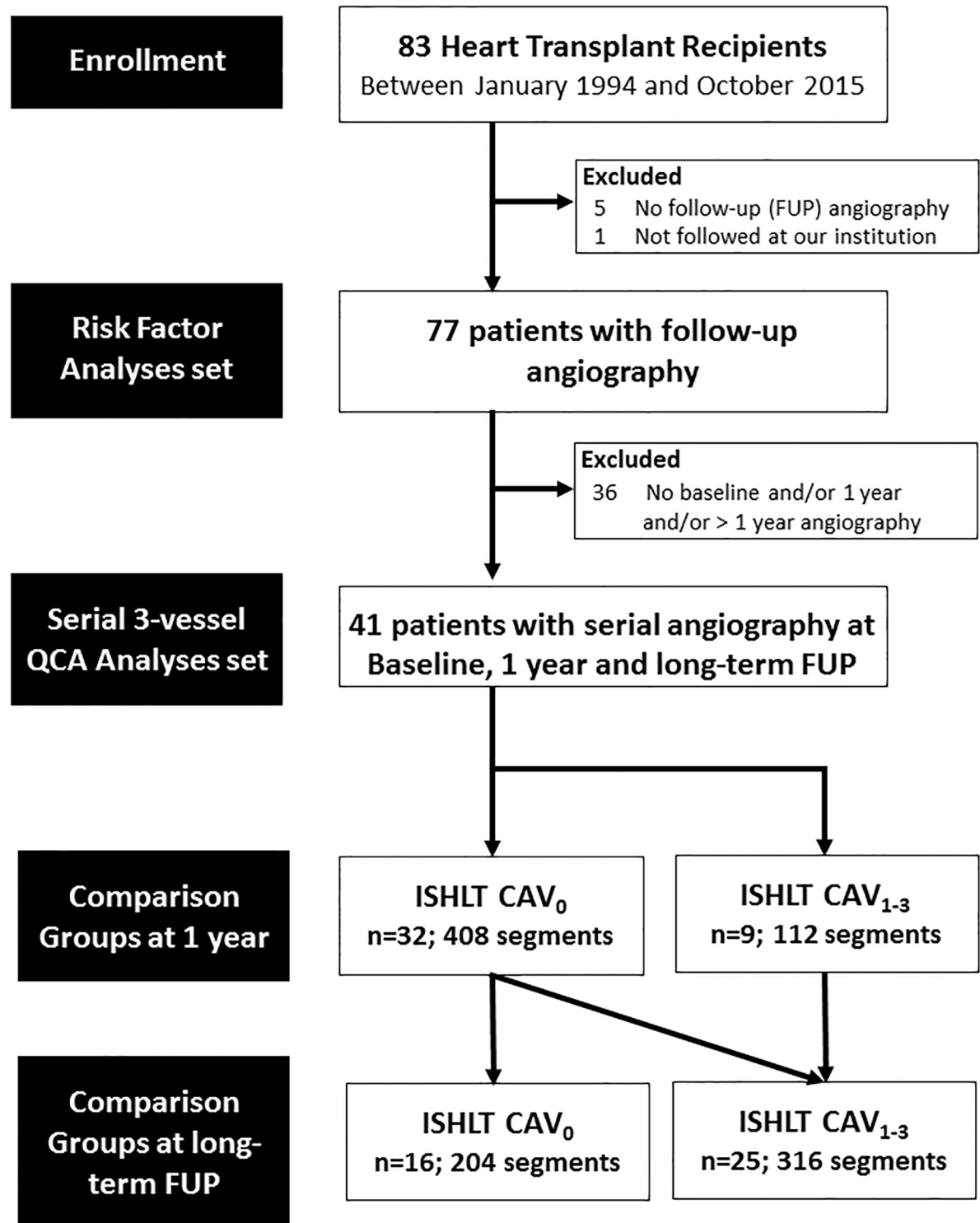


Fig 1. Flow chart. CAV indicates cardiac allograft vasculopathy; FUP, follow-up; QCA, quantitative coronary angiography; ISHLT, International Society of Heart and Lung Transplantation.

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Coronary angiography assessment

CAV was classified by a retrospective review of all coronary angiographic and echocardiographic studies at 1 year, and at latest angiographic follow-up after HTx, based on the ISHLT guidelines [5] as follows: ISHLT-CAV₀ indicates no visually detectable angiographic lesion; ISHLT-CAV₁ (mild) indicates angiographic left main <50%, or primary vessel with a maximum lesion of <70%, or any branch stenosis <70% (including diffuse narrowing) without allograft dysfunction; ISHLT-CAV₂ (moderate) indicates angiographic left main <50%, a single primary vessel $\geq 70\%$, or isolated branch stenosis $\geq 70\%$ in branches of 2 systems, without allograft dysfunction; and ISHLT-CAV₃ (severe) indicates angiographic left main $\geq 50\%$, or two or more primary vessels with $\geq 70\%$ stenosis, or isolated branch stenosis $\geq 70\%$ in all 3 systems, or ISHLT-CAV₁ or ISHLT-CAV₂ with allograft dysfunction (defined as left ventricular ejection fraction $\leq 45\%$ with the presence of regional wall motion abnormalities). For the current analysis, we defined the presence of CAV as a status \geq ISHLT-CAV₁ and we grouped the study population in ISHLT-CAV₀ and ISHLT-CAV₁₋₃. We conducted follow-up and angiographic assessment until April 2016.

Quantitative coronary angiography assessment

Standard biplane angiographic images were obtained after administration of intra-coronary nitroglycerin (100–300 μ g) with a frame rate of 15/second. Each coronary segment was recorded in at least two orthogonal views. All angiographies were analyzed at the angiographic core laboratory at the University Hospital of Bern by two independent clinicians (CZ, KY), and a third clinician in case of disagreement (LR), who were blinded to any clinical data. All three major epicardial vessels including all side branches with a reference vessel diameter (RVD) of >1.5mm were assessed by QCA at baseline, at 1-year, and at long-term angiographic follow-up after HTx by using similar projections whenever possible. For this purpose, segments of the coronary vessels were divided into subsegments according to the modified American Heart Association/American College of Cardiology (AHA/ACC) [9] classification (Fig 2). QCA analysis was done by using the QangioXA version 7.3 (Medis Medical Imaging Systems, Leiden, the Netherlands). Minimal lumen diameter (MLD), RVD, segment length and maximal percent diameter stenosis [%DS, calculated by $(1 - \text{MLD}/\text{RVD}) \times 100$] within the subsegments were assessed. In the case of interim revascularization, the revascularized vessel was analyzed prior to the revascularization, whereas the non-revascularized vessel segments were analyzed at the long-term angiographic follow-up. The change of all variables was derived for each segment as outcome (long-term angiographic follow-up and 1 year follow-up) minus outcome (1 year follow-up and baseline). The primary angiographic endpoint was mean change in MLD and %DS. Analysis was performed to determine the MLD and %DS change within the two groups (ISHLT-CAV₀ vs. ISHLT-CAV₁₋₃) 1 year after HTx and at long-term angiographic follow-up.

Immunosuppression therapy and rejection score

The induction immunosuppressive therapy consisted of azathioprine 5 mg/kg and 1000 mg of methylprednisolone. Antithymocyte globulin (4–5 mg/kg) therapy was introduced within 12 hours post-transplant and thereafter tailored by CD3 cell count. A calcineurin inhibitor (CNI) based immunosuppressive therapy was initiated as a standard maintenance therapy. In case of progressive impairment of renal function, everolimus (ERL) was initiated already within the first month after HTx. Other reasons for switching to ERL were progressive CAV and skin malignancy or side effects. Switching to ERL therapy was a decision that was left to the

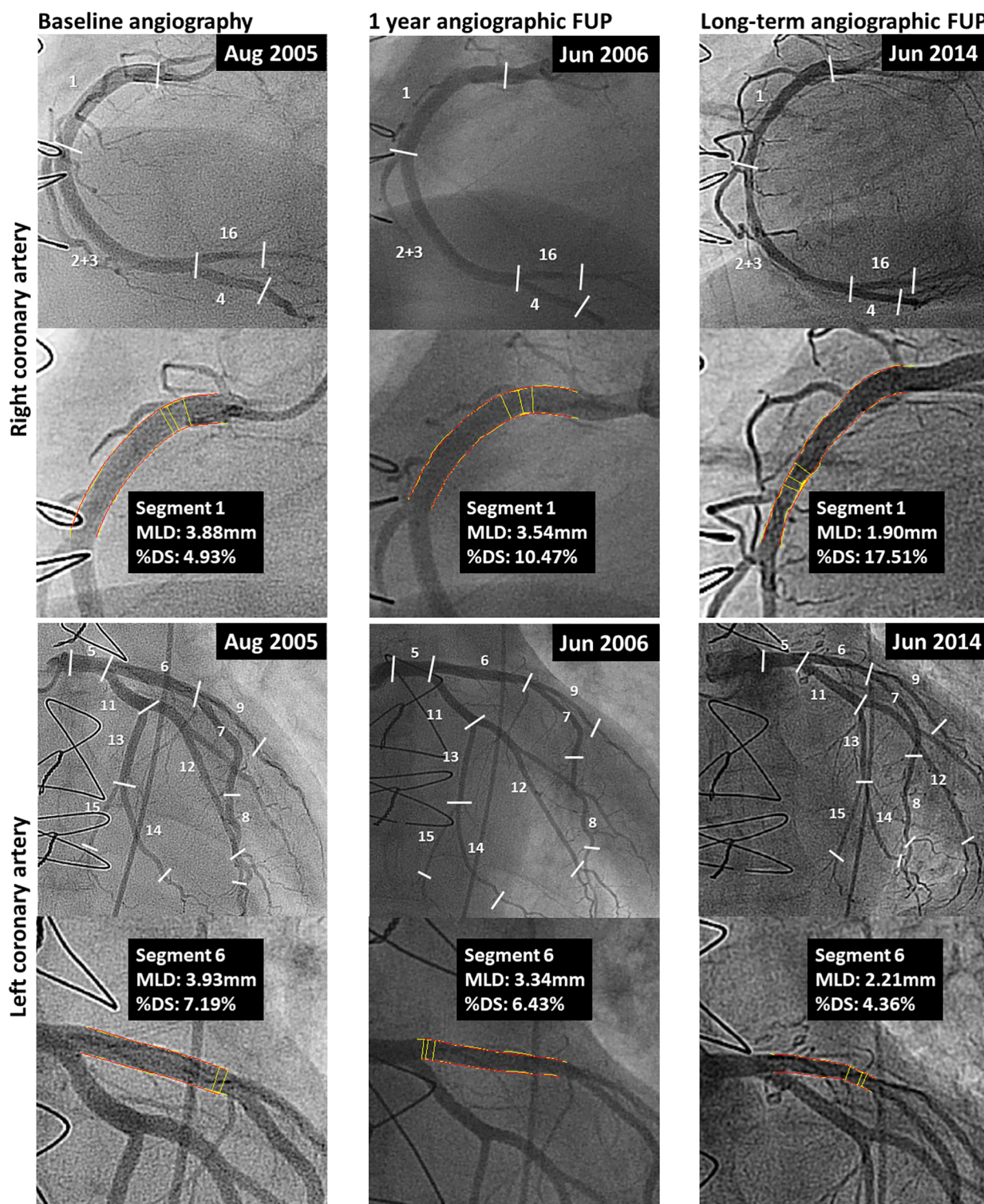


Fig 2. Serial 3-vessel quantitative coronary angiography analysis. This figure shows the serial quantitative coronary angiography analysis within matched regions of all coronary artery segments at baseline, at 1 year follow-up, and at latest available angiographic follow-up. Coronary artery segments were classified according to the modified AHA/ACC classification. MLD indicates minimal lumen diameter; %DS, percent diameter stenosis.

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discretion of the experienced transplant cardiologist. The general targeted ERL trough concentration (C_0) was 6–8 ng/mL and 8–14 ng/mL for tacrolimus (TAC) with a concomitant therapy of prednisone and mycophenolate mofetil (MMF) or azathioprine. Endomyocardial biopsy (EMB) specimens were graded according to the 2005 ISHLT classification for acute cellular rejection (ACR) as 0R = 0, 1R = 1, 2R = 2, and 3R = 3 [13]. Severe Total Rejection Score (TRS) was defined as number of ACR \geq 2R divided by the total numbers of biopsies. Antibody-mediated rejection (AMR) was diagnosed in the EMB specimens according to standardized histopathologic signs of AMR and, if indicated, with immunohistochemistry to confirm AMR. No immunohistochemistry positive EMBs or allograft rejections with hemodynamic compromise were observed.

Clinical and demographic data

Clinical and angiographic data were retrieved by a retrospective review of all patient charts or database. The cardiovascular risk factors were monitored according to local guidelines. Hypertension was defined as systolic blood pressure \geq 140 mmHg and/or diastolic blood pressure \geq 90 mmHg. Hypercholesterolemia was defined as total cholesterol \geq 5.2 mmol/L. MDRD (Modification of Diet in Renal Disease) glomerular filtration rate (shown as GFR; creatinine shown as Cr) was calculated by using the following formula: $GFR = 186 \times \text{Serum-Cr}^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ if patient was female})$.

Acetylsalicylic acid (ASA) therapy was continued after HTx in most patients with previous CAD or as indicated after percutaneous coronary intervention (PCI). After HTx, all patients received statin therapy. Ezetimibe was added in case of insufficient lipid lowering effect. In case of high-risk cytomegalovirus (CMV) serologic status (*i.e.*, donor positive and recipient negative), HTx recipients received ganciclovir or valganciclovir CMV prophylaxis adapted to individual kidney function for 6 months. Pre-emptive CMV treatment was given in all other patients throughout the follow-up period.

Statistical analysis

Continuous variables for patient-level data are expressed as median with interquartile range or mean \pm standard deviation (SD). Categorical variables are expressed as number and percentage. Patients were stratified into two groups according to the presence or absence of ISHLT-CAV at 1 year and at latest angiographic follow-up. Comparisons between the groups regarding risk factors of CAV were performed by using the χ^2 test. All hypotheses tested were 2-tailed and a p -value < 0.05 was considered statistically significant.

QCA outcomes were recorded for segments per patient at baseline, at 1 year, and at long-term angiographic follow-up. For each segment, we computed the absolute change from baseline to 1-year, from baseline to long-term angiographic follow-up, and from 1-year to long-term angiographic follow-up. Patient-level outcomes and their changes were generated by using generalized linear mixed effect model. Wilcoxon rank sum test was used to compare the changes of parameters between baseline, 1 year, and long-term angiographic follow-up. To assess the subsegment-level data of the QCA analysis and its associations of all clinical variables, including the ISHLT-CAV grading score, a linear regression analysis was used that took into account a random effect of patient-level data.

Statistical analyses were performed with the use of R version 3.2.3 (R Foundation for Statistical Computing).

Results

Baseline characteristics

The donor and recipient characteristics of the study cohort are summarized in [Table 1](#). The study population consisted of 77 patients (57 males), with a mean recipient age at HTx of 46.6 (± 15.7) years. ERL-based therapy was administered in 41 (53%) and TAC-based therapy in 36 (47%) patients. The leading cause of HTx was dilated cardiomyopathy ($n = 36$; 47%), followed by ischemic cardiomyopathy ($n = 27$; 35%). During follow-up, 14 patients underwent PCI. Thirteen underwent PCI due to CAV and 1 patient due to fistula from the left anterior descending artery to the right ventricle. No patient had echocardiographic allograft dysfunction (defined as LVEF $\leq 45\%$ with the presence of regional wall motion abnormalities) at latest follow-up.

Serial 3-vessel quantitative coronary angiography analyses

A total of 520 native coronary artery segments (12.7 segments per patient) at baseline (median 0.14 [0.12 to 0.16] years after HTx) were matched with the corresponding segments at 1 year follow-up (median 1.17 [1.14 to 1.20] years after HTx) and at long-term angiographic follow-up (median 8.61 [8.24 to 8.99] years after HTx). [Fig 3](#) shows the overall mean change of MLD and %DS from baseline to 1 year follow-up, and from 1 year follow-up to long-term angiographic follow-up. MLD decreased non-significantly from baseline to 1 year follow-up ($\Delta -0.04$ mm/year [95% CI -0.17 to -0.08], $p = 0.49$), and significantly from 1 year follow-up to long-term angiographic follow-up ($\Delta -0.08$ mm/year [95% CI -0.11 to -0.05], $p < 0.001$). %DS increased significantly from baseline to 1 year follow-up ($\Delta +0.96\%$ /year [95% CI 0.04 to 1.88], $p = 0.041$), and from 1 year follow-up to long-term angiographic follow-up ($\Delta +0.61\%$ /year [95% CI 0.33 to 0.88], $p < 0.001$).

[Table 2](#) represents the serial 3-vessel QCA results between the ISHLT-CAV groups. On coronary angiography at 1 year, 32 patients (78%) were graded as ISHLT-CAV₀, whereas 9 patients (22%) were graded as ISHLT-CAV₁₋₃ [ISHLT CAV₁, $n = 9$ (100%); ISHLT-CAV₂, $n = 0$ (0%); ISHLT-CAV₃, $n = 0$ (0%)]. At long-term angiographic follow-up, 16 patients (39%) were graded as ISHLT-CAV₀, whereas 25 patients (61%) were graded as ISHLT-CAV₁₋₃ [ISHLT-CAV₁, $n = 18$ (72%); ISHLT-CAV₂, $n = 7$ (28%); ISHLT-CAV₃, $n = 0$ (0%)]. A reduction in MLD was observed within both groups between baseline and 1 year follow-up, and between baseline and long-term angiographic follow-up. The reduction in MLD between the two groups did not differ at 1 year (ISHLT-CAV₀: median -0.04 mm [IQR -0.10 to 0.02] vs. ISHLT-CAV₁₋₃: median -0.07 mm [IQR -0.12 to -0.02], $p = 0.8$) and during long-term angiographic follow-up (ISHLT-CAV₀: median -0.49 mm [IQR -0.54 to -0.43] vs. ISHLT-CAV₁₋₃: median -0.40 mm [IQR -0.44 to -0.35], $p = 0.4$) ([Fig 4](#)). Patients who developed ISHLT-CAV₁₋₃ during long-term follow-up showed a significantly higher baseline %DS (ISHLT-CAV₀: median 13.6% [IQR 13.2 to 14.1] vs. ISHLT-CAV₁₋₃: median 15.9% [IQR 15.6 to 16.3], $p = 0.01$), and at long-term angiographic follow-up %DS (ISHLT-CAV₀: median 15.9% [IQR 15.3 to 16.6] vs. ISHLT-CAV₁₋₃: median 21.0% [20.5 to 21.5], $p < 0.001$). The increase in %DS between baseline and long-term angiographic follow-up was significantly higher in the ISHLT-CAV₁₋₃ group (ISHLT-CAV₀: median 2.3% [IQR 1.7 to 2.9] vs. ISHLT-CAV₁₋₃: median 5.1% [IQR 4.6 to 5.5], $p = 0.02$).

Cardiac allograft vasculopathy risk factors

[Table 3](#) shows baseline clinical characteristics according to the presence or absence of ISHLT-CAV₁₋₃ at latest angiographic follow-up. On latest coronary angiography, 39 patients (51%)

Table 1. Baseline clinical characteristics.

	HTx recipients (n = 77)
Recipient age at transplantation (years)	46.6 ± 15.7
Donor age (years)	43.4 ± 13.2
Recipient (male)	57 (74)
Primary cause of HTx	
Dilated cardiomyopathy	36 (47)
Ischemic cardiomyopathy	27 (35)
Arrhythmogenic right ventricular cardiomyopathy	5 (6)
Hypertrophic cardiomyopathy	2 (3)
Valvular heart disease	2 (3)
Other causes*	5 (6)
Cardiac risk factors	
BMI (kg/m ²)	24.6 ± 4.7
Hypertension	30 (39)
Systolic blood pressure (mmHg)	130 ± 17
Diastolic blood pressure (mmHg)	78 ± 12
HbA1c	5.8 ± 0.7
Active smoker	9 (12)
eGFR (mL/min)	65.2 ± 27.3
CMV serology, donor positiv/recipient negativ	21 (27)
CMV infection	29 (38)
Severe TRS ≥ 2R	0.040 ± 0.052
Lipids	
Hypercholesterolemia	37 (48)
Total cholesterol (mmol/L)	5.3 ± 1.5
HDL cholesterol (mmol/L)	1.6 ± 0.5
LDL cholesterol (mmol/L)	3.2 ± 1.2
Triglycerides (mmol/L)	2.2 ± 1.5
Medication	
ACEi / ARB	54 (70)
Beta Blocker	21 (27)
Calcium channel blocker	24 (31)
Aspirin or Clopidogrel	51 (66)
Statin	77 (100)
Ezetimibe	19 (25)
Immunosuppressive Regimes	
Everolimus, MMF, Prednisone	24 (31)
Everolimus, MMF	13 (17)
Everolimus, Prednisone	3 (4)
Everolimus, AZA, Prednisone	1 (1)
Tacrolimus, MMF	18 (23)
Tacrolimus, MMF, Prednisone	12 (16)
Tacrolimus, Prednisone	4 (5)
Tacrolimus, AZA	2 (3)
Echocardiography	
LVEF (%)	62 ± 6

Categorical variables are shown as number (%). Continuous variables are shown as mean ± SD
HTx, heart transplantation; BMI, body mass index; eGFR, estimated glomerular filtration rate; CMV, cytomegalovirus; TRS, total rejection score; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; MMF, Mycophenolate mofetil; AZA, Azathioprin; LVEF, left ventricular ejection fraction

*Other causes: congenital heart disease (1 patient), giant cell myocarditis (1 patient), Naxos-Syndrome (1 patient), myofibrillar myopathy (1 patient), biventricular heart failure of unknown etiology (1 patient)

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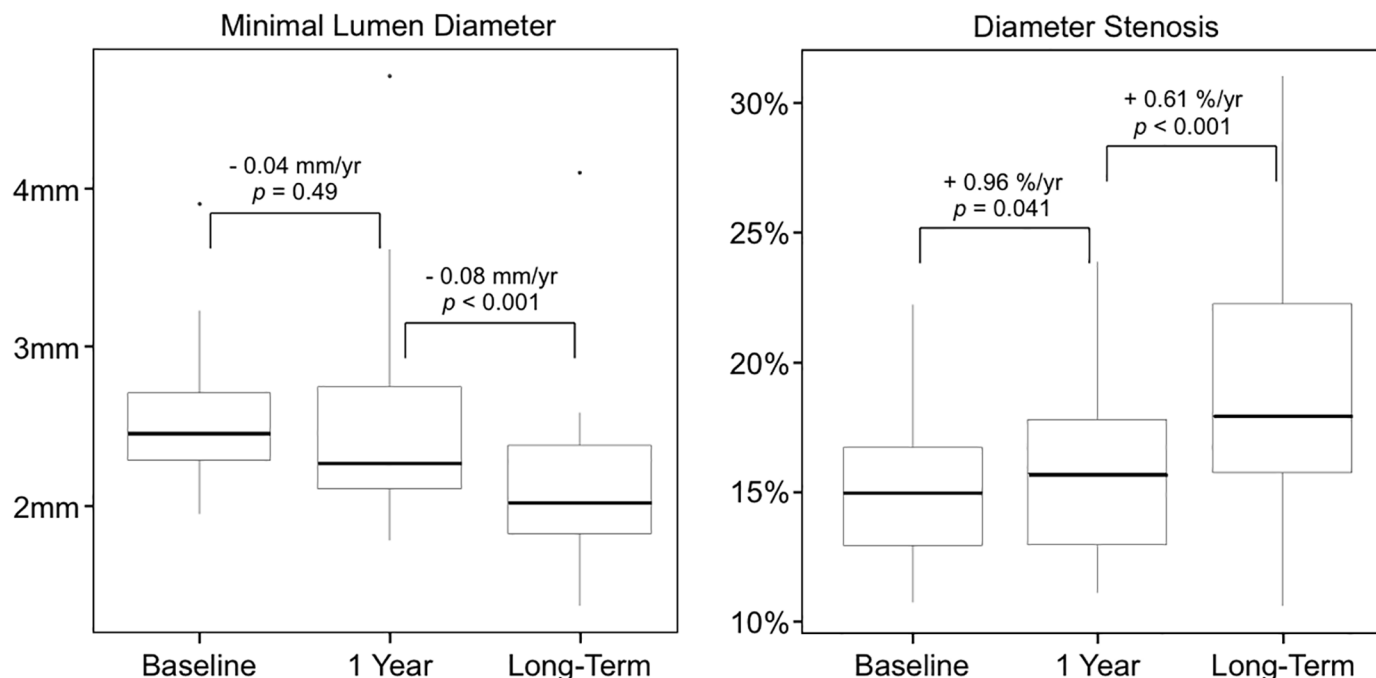


Fig 3. Serial 3-vessel quantitative coronary angiography analysis. Box-plot representation of minimal lumen diameter and maximal percent diameter stenosis at baseline (median 0.14 years [0.12 to 0.16], at 1 year (median 1.17 years [1.14 to 1.20]) and at long-term angiographic follow-up (median 8.61 years [8.24 to 8.99]) after HTx. Lower and upper box edges are the quartiles and thick line is the median.

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were graded as ISHLT-CAV₀, whereas 38 patients (49%) were graded as ISHLT-CAV₁₋₃ [CAV₁, $n = 25$ (66%); CAV₂, $n = 12$ (32%); CAV₃, $n = 1$ (3%)]. ISHLT-CAV₁₋₃ was significantly associated with eight factors: higher recipient age (45.0 years [26.0–54.0] vs. 55.5 years [42.5–61.5]; $p = 0.01$); higher donor age (38.5 years [31.0–49.7] vs. 49.0 years [39.0–57.5]; $p = 0.009$); CAD before HTx (9 (23%) vs. 18 (47%); $p = 0.046$); lower eGFR (70.2 ml/min [52.1–87.4] vs. 54.8 ml/min [39.4–66.8]; $p = 0.005$); higher LDL cholesterol (2.5 mmol/l [2.1–3.7] vs. 3.2 mmol/l [2.5–3.9]; $p = 0.08$); higher triglyceride (1.5 mmol/l [1.1–2.0] vs. 2.3 mmol/l [1.3–3.8]; $p = 0.01$); severe TRS ≥ 2 (0.00 [0.00–0.05] vs. 0.05 [0.00–0.09]; $p = 0.005$); and male donor (24 (62%) vs. 32 (84%); $p = 0.07$). Higher max %DS was significantly associated with five factors: higher recipient age (48.0 years [22.0–56.5] vs. 53.0 years [41.2–60.0]; $p = 0.04$); higher HbA1c (5.5 [5.3–5.8] vs. 5.8 [5.6–6.1]; $p = 0.004$); lower eGFR (68.2 ml/min [56.9–89.2] vs. 53.8 ml/min [39.4–69.3]; $p = 0.002$); higher LDL cholesterol 2.5 mmol/l [2.1–3.6] vs. 3.2 mmol/l [2.5–4.0]; $p = 0.09$); and severe TRS ≥ 2 (0.00 [0.00–0.06] vs. 0.04 [0.00–0.09]; $p = 0.03$). There were no significant differences between the two groups regarding the adherence to immunosuppressive medications and cardiovascular medication including ERL, TAC, MMF, azathioprine, prednisone, ACE inhibitor/ARB, statin, and ezetimibe.

Discussion

The four main findings of this study can be summarized as follows. First, patients without visual signs of CAV during follow-up showed similar MLD loss compared with ISHLT-CAV₁₋₃ patients. Secondly, MLD loss between baseline and 1-year after HTx did not predict CAV progression beyond 1 year. Thirdly, baseline %DS was higher in patients who were developing ISHLT-CAV₁₋₃ at latest angiographic follow-up. Fourth, risk factors of CAV according to the ISHLT-CAV classification were in line with the risk factors associated with the QCA analyses.

Table 2. QCA analysis of serial angiography at baseline, 1 year and long-term follow-up after heart transplantation.

	All patients	At 1 year		<i>p</i> -value	At long-term follow-up		<i>p</i> -value
		ISHLT-CAV ₀	ISHLT-CAV ₁₋₃		ISHLT-CAV ₀	ISHLT-CAV ₁₋₃	
Number of patients	41	32	9		16	25	
Number of segments	520	408	112		204	316	
Number of segments per patient	12.7	12.8	12.4		12.8	12.6	
Reference Vessel Diameter (mm)							
Baseline	3.10 [3.06 to 3.15]	3.10 [3.05 to 3.15]	3.12 [3.02 to 3.22]	0.91	3.13 [3.06 to 3.21]	3.09 [3.02 to 3.15]	0.74
1-year	3.04 [2.97 to 3.12]	2.97 [2.89 to 3.06]	3.29 [3.13 to 3.44]	0.26	3.09 [2.97 to 3.21]	3.01 [2.91 to 3.10]	0.72
Latest FUP	2.67 [2.61 to 2.72]	2.65 [2.58 to 2.71]	2.73 [2.61 to 2.85]	0.67	2.58 [2.50 to 2.67]	2.72 [2.65 to 2.79]	0.43
Segment Length (mm)							
Baseline	36.1 [35.4 to 36.9]	36.0 [35.1 to 36.8]	36.6 [35.0 to 38.3]	0.81	34.9 [33.7 to 36.1]	36.9 [35.9 to 37.8]	0.41
1-year	36.7 [36.0 to 37.4]	36.1 [35.3 to 36.9]	38.8 [37.3 to 40.3]	0.31	34.6 [33.5 to 35.7]	38.0 [37.1 to 38.9]	0.12
Latest FUP	33.7 [33.1 to 34.3]	33.6 [33.0 to 34.3]	33.8 [32.5 to 35.1]	0.93	31.5 [30.6 to 32.4]	35.1 [34.3 to 35.8]	0.06
Total Segment Length (mm)							
Baseline	456.4 [446.7 to 466.1]	456.7 [445.6 to 467.9]	455.1 [434.1 to 476.1]	0.96	444.9 [429.2 to 460.5]	463.8 [451.2 to 476.3]	0.53
1-year	463.7 [454.8 to 472.7]	459.0 [448.8 to 469.2]	480.5 [461.2 to 499.8]	0.51	441.2 [427.0 to 455.4]	478.1 [466.8 to 489.5]	0.17
Latest FUP	426.4 [418.1 to 434.7]	428.0 [418.5 to 437.5]	420.6 [402.6 to 438.5]	0.8	401.6 [388.6 to 414.7]	442.2 [431.8 to 452.6]	0.11
Minimal Lumen Diameter (mm)							
Baseline	2.53 [2.49 to 2.57]	2.55 [2.50 to 2.59]	2.48 [2.39 to 2.56]	0.64	2.61 [2.54 to 2.67]	2.48 [2.43 to 2.54]	0.34
1-year	2.47 [2.41 to 2.53]	2.44 [2.37 to 2.51]	2.59 [2.46 to 2.71]	0.5	2.56 [2.47 to 2.66]	2.41 [2.34 to 2.49]	0.41
Latest FUP	2.10 [2.05 to 2.14]	2.09 [2.04 to 2.15]	2.11 [2.01 to 2.21]	0.94	2.11 [2.04 to 2.19]	2.09 [2.03 to 2.15]	0.87
Δ* in MLD (1 year-BL)	-0.06 [-0.10 to -0.02]	-0.11 [-0.15 to -0.06]	0.10 [0.02 to 0.19]	0.15	-0.04 [-0.10 to 0.02]	-0.07 [-0.12 to -0.02]	0.8
Δ* in MLD (FUP-BL)	-0.43 [-0.47 to -0.40]	-0.45 [-0.49 to -0.41]	-0.38 [-0.45 to -0.30]	0.56	-0.49 [-0.54 to -0.43]	-0.40 [-0.44 to -0.35]	0.4
Diameter Stenosis (%)							
Baseline	15.0 [14.7 to 15.3]	14.7 [14.4 to 15.0]	16.3 [15.7 to 17.0]	0.13	13.6 [13.2 to 14.1]	15.9 [15.6 to 16.3]	0.01
1-year	16.0 [15.67 to 16.4]	15.6 [15.2 to 16.0]	17.6 [16.9 to 18.4]	0.12	15.1 [14.5 to 15.6]	16.7 [16.2 to 17.1]	0.16
Latest FUP	19.0 [18.6 to 19.5]	18.2 [17.7 to 18.8]	21.9 [20.9 to 22.9]	0.04	15.9 [15.3 to 16.6]	21.0 [20.5 to 21.5]	<0.01
Δ* in %DS (1 year-BL)	1.0 [0.7 to 1.3]	0.9 [0.6 to 1.3]	1.3 [0.7 to 2.0]	0.72	1.4 [1.0 to 1.9]	0.7 [0.3 to 1.1]	0.43
Δ* in %DS (FUP-BL)	4.0 [3.6 to 4.4]	3.6 [3.1 to 4.0]	5.6 [4.8 to 6.4]	0.15	2.3 [1.7 to 2.9]	5.1 [4.6 to 5.5]	0.02

Patient-level outcomes derived as the median (mm) and interquartile range. P-values using generalized linear mixed effect model taking random effects of patient-level data into account. QCA indicates quantitative coronary angiography; BL, baseline; FUP, follow-up; MLD, minimal lumen diameter; %DS, percent diameter stenosis; Δ, difference;

*Change was derived at the level of segments.

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Use of serial three-vessel QCA to assess cardiac allograft vasculopathy progression

The present study made it possible to assess short- and long-term progression of CAV with the current standard of visual ISHLT-CAV classification and the serial 3-vessel QCA analyses within all segments of the three coronary arteries. The advantage of using QCA is that it detects subtle changes of luminal narrowing. IVUS studies have shown that approximately 80% of patients already show a thickening of the coronary intima within the first year after HTx [9]. Of note, most of those patients generate the intimal thickening without angiographical signs of CAV. Importantly, the most rapid rate of intimal thickening occurs during the first

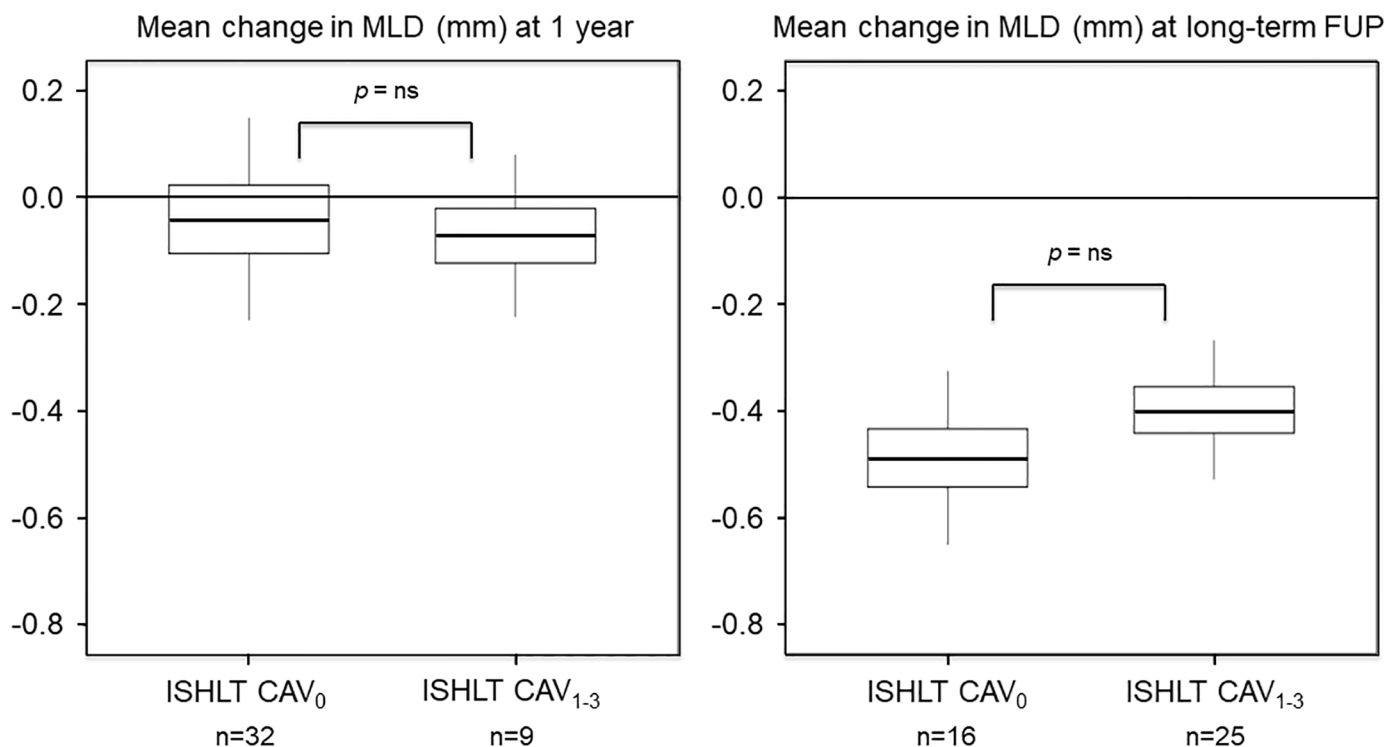


Fig 4. Quantitative angiographic analysis. Box-plot representation of the per-patient mean angiographic change in minimal lumen diameter (minimal lumen diameter 1 year—minimal lumen diameter baseline and minimal lumen diameter latest angiographic follow-up—minimal lumen diameter baseline) from coronary artery segments that were serially assessed and matched. The analysis is stratified according to absence ($n = 16$) or presence ($n = 25$) of ISHLT-CAV at 1 year, and at latest angiographic follow-up (median 8.61 years [8.24 to 8.99]). Lower and upper box edges are the quartiles and thick line is the median. A horizontal reference line at change = 0 is drawn.

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year after HTx, followed by a slower rate of intimal thickening. Furthermore, a rapid progression of maximal intimal thickness of >0.5 mm during the first year after HTx has been shown to be predictive of angiographic CAV after five years [14] and of increased risk of all-cause mortality and myocardial infarction [10]. Due to a lack of standardized predictive value of lumen change between baseline and 1 year, such cut-off values are not available for QCA in the current analysis. In contrast to our expectation, the reduction in MLD from baseline to long-term angiographic follow-up did not differ between patients with ISHLT-CAV₀ and ISHLT-CAV₁₋₃ (ISHLT-CAV₀: median -0.49 mm [IQR -0.54 to -0.43] vs. ISHLT-CAV₁₋₃: median -0.40 mm [IQR -0.44 to -0.35], $p = 0.4$). A failure in QCA to detect a significant lumen loss between baseline and 1 year, as an indirect indicator of the vessel wall thickening, might be influenced by an expansion of the external elastic membrane leading to a preservation of the luminal area without angiographic signs of CAV [15,16]. Hence, since angiography only depicts the lumen contour, QCA could not identify early intimal thickening by narrowing of the lumen area in patients who developed ISHLT-CAV₁₋₃ during long-term follow-up. Furthermore, the similarity in the lumen loss between the two groups during long-term follow-up might be caused by a diffuse longitudinal and concentric narrowing of the coronary artery vessel wall that decreases MLD even though no angiographic signs of CAV were apparent. Furthermore, RVD decreased over time in a similar way like MLD, which indicates that the lumen narrowed in the coronary artery tree without visual plaque stenosis. This pathophysiological mechanism of CAV progression has been previously described in histopathology studies [17–19]. Hence, diffuse concentric intimal thickening and narrow points of constriction may be

Table 3. Baseline clinical characteristics in patients with and without ISHLT-CAV at latest follow-up.

	ISHLT-CAV ₀ at latest follow-up n = 39	ISHLT-CAV ₁₋₃ at latest follow-up n = 38	p-value	Lower max %DS n = 39	Higher max %DS n = 38	p-value
Recipient profile						
Age (years)	45.0 [26.0–54.0]	55.5 [42.5–61.5]	0.01	48.0 [22.0–56.5]	53.0 [41.2–60.0]	0.04
Male	28 (72)	29 (76)	0.85	27 (69)	30 (79)	0.48
Body mass index, kg/m ²	24.2 [22.5–26.4]	24.1 [20.6–27.9]	0.83	23.81 [21.5–26.2]	24.3 [21.6–28.1]	0.29
CAD before HTx	9 (23)	18 (47)	0.046	11 (28)	16 (42)	0.3
Hypertension	15 (38)	15 (39)	1	13 (33)	17 (45)	0.43
HbA1c (%)	5.6 [5.4–5.8]	5.7 [5.4–6.1]	0.12	5.5 [5.3–5.8]	5.8 [5.6–6.1]	0.004
Active Smoker	5 (13)	4 (11)	1	4 (10)	5 (13)	0.97
eGFR (ml/min)	70.2 [52.1–87.4]	54.8 [39.4–66.8]	0.005	68.2 [56.9–89.2]	53.8 [39.4–69.3]	0.002
Hypercholesteremia	16 (41)	21 (55)	0.31	18 (46)	19 (50)	0.91
Total cholesterol (mmol/L)	4.5 [4.0–5.8]	5.2 [4.5–6.2]	0.13	4.9 [4.0–5.8]	5.2 [4.5–6.2]	0.19
HDL cholesterol (mmol/L)	1.6 [1.2–2.1]	1.5 [1.2–1.8]	0.49	1.5 [1.2–1.9]	1.6 [1.3–1.9]	0.57
LDL cholesterol (mmol/L)	2.5 [2.1–3.7]	3.2 [2.5–3.9]	0.08	2.5 [2.1–3.6]	3.2 [2.5–4.0]	0.09
Triglyceride (mmol/L)	1.5 [1.1–2.0]	2.3 [1.3–3.8]	0.01	1.8 [1.1–2.4]	2.0 [1.3–2.8]	0.52
CMV, donor pos./ recipient neg.	8 (21)	13 (34)	0.27	10 (25)	11 (29)	0.94
CMV infection	12 (31)	17 (45)	0.3	12 (31)	17 (45)	0.3
Severe TRS ≥ 2R	0.00 [0.00–0.05]	0.05 [0.00–0.09]	0.005	0.00 [0.00–0.06]	0.04 [0.00–0.09]	0.03
LVEF-FUP (%)	65.0 [60.0–65.0]	60.0 [60.0–65.0]	0.07	65.0 [60.0–65.0]	60.0 [60.0–65.0]	0.19
LVEDP (mmHg)	12.5 [8.5–17.0]	11.5 [6.0–15.0]	0.25	13.0 [8.0–17.5]	11.0 [6.0–14.0]	0.13
Donor profile						
Age (years)	38.5 [31.0–49.7]	49.0 [39.0–57.5]	0.009	42.0 [33.0–52.8]	48.0 [36.2–55.5]	0.16
Male	24 (62)	32 (84)	0.07	25 (64)	31 (82)	0.19

Values shown are median [lower to upper quartile] or number (%), $p < 0.05$ was considered statistically significant; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoproteins; LDL, low-density lipoproteins; MLD, minimal lumen diameter; RVD, reference vessel diameter; CMV, cytomegalovirus; TRS, total rejection score; LVEF, left ventricular ejection fraction; FUP, follow-up, LVEDP, left ventricular end diastolic pressure.

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overlooked with coronary angiography, and applying the ISHLT classification might underestimate the CAV progression since it is mainly defined by stenotic plaques. As shown in our study, %DS expressed the vascular plaque stenosis in accordance to the ISHLT-CAV classification, which shows a significant difference in %DS at baseline and during long-term angiographic follow-up between the ISHLT-CAV groups.

Arora et.al. [7] demonstrated a significantly reduced CAV progression, and significantly less coronary intimal thickening, in patients treated with *de novo* everolimus-based therapy and early calcineurin inhibitor withdrawal, compared to the calcineurin inhibitor control group irrespective of donor disease in a matched IVUS examination at baseline and 12 months after HTx. In our cohort of 41 patients, 53% of the patients received everolimus-based and calcineurin inhibitor-free immunosuppressive therapy at latest angiographic follow-up, 42% of whom switched therapies within the first month after HTx. Hence, the use of potent immunosuppressive drugs in our cohort such as mycophenolate mofetil and everolimus might have decelerated CAV progression. In our cohort, including matched serial three vessel QCA analyses, 78% had no visual signs of CAV one year after HTx, a finding possibly influenced by the early everolimus therapy after HTx. However, during long-term angiographic follow-up there was no significant difference in the presence of CAV between the everolimus-based group compared with the tacrolimus-based group. Furthermore, the prevalence of CAV detected by

coronary angiography at 1, 5, and 10 years after HTx is approximately 10–20%, 35–50%, and 50–60% [9], respectively. This finding is in line with our data, which report the prevalence of CAV at 1 year, and at a mean follow-up time of 9 years in 22% and 61% of patients, respectively. Of note, only 1 patient had visual sign of donor transmitted CAD at baseline.

Risk factors of cardiac allograft vasculopathy

Several risk factors have been linked with CAV development including increasing donor age, donor history of hypertension, pre-transplant CAD, and cardiovascular risk factors [20]. To further validate the QCA method in our cohort, we assessed the risk factors of CAV with %DS and the ISHLT-CAV classification. Risk factors of CAV associated with maximal %DS and the ISHLT-CAV classification were similar, including recipient age, HbA1c, eGFR, and severe TRS $\geq 2R$. Even though QCA is a more sensitive method than the visual assessment in identifying subtle changes of the coronary artery tree, it could not identify additional risk factors compared to the ISHLT-CAV classification. Surprisingly, the ISHLT-CAV classification additionally detected donor age, donor male, CAD before HTx, and triglycerides as CAV risk factors. Delgado et al. [21] also showed that donor age, CMV infection, and presence of cellular acute rejection $\geq 2R$ are predictors of CAV. In our study, CMV infection was not associated with CAV, which is in conflict with recent large cohort studies [21,22]. In a review by Braga et al. [23] the inconsistency of the CAV risk factors was suggested to be influenced by different CAV assessment methods, classification, and observation time.

The results of our single-centre retrospective study have to be interpreted in the light of some limitations. First, the lack of serial angiographies resulted in a limited sample size. Second, patients with more side branches, but with a reference diameter $>1.5\text{mm}$, could have a lower MLD not reflective of CAV. Nevertheless, baseline MLD between the two groups showed no significant difference in MLD and RVD, thus representing comparable groups. Furthermore, there was no significant difference between the two groups regarding the measured segments per patient and segment length. Consequently, the results should be regarded as exploratory.

Conclusions

Mean lumen diameter loss between baseline and 1-year after heart transplantation as assessed by QCA in all coronary artery segments did not predict CAV progression in the long-term. There was no difference in MLD loss over time within the coronary tree between ISHLT-CAV₀ and ISHLT-CAV₁₋₃ patients as assessed by QCA, suggesting a similar progression of CAV independently of the ISHLT-CAV classification.

Supporting information

S1 Data.
(XLSX)

S2 Data.
(XLSX)

Author Contributions

Conceptualization: Christian Zanchin, Kyohei Yamaji, Thomas Zanchin, Stephan Windecker, Lorenz Räber, Vilborg Sigurdardottir.

Data curation: Christian Zanchin, Carolin Rogge, Vilborg Sigurdardottir.

Formal analysis: Christian Zanchin, Vilborg Sigurdardottir.

Investigation: Vilborg Sigurdardottir.

Methodology: Kyohei Yamaji, Carolin Rogge, Lorenz Räber, Vilborg Sigurdardottir.

Project administration: Lorenz Räber, Vilborg Sigurdardottir.

Supervision: Lorenz Räber, Vilborg Sigurdardottir.

Visualization: Christian Zanchin, Kyohei Yamaji, Thomas Zanchin, Yasushi Ueki, Lorenz Räber, Vilborg Sigurdardottir.

Writing – original draft: Christian Zanchin.

Writing – review & editing: Christian Zanchin, Kyohei Yamaji, Carolin Rogge, Dorothea Lesche, Thomas Zanchin, Yasushi Ueki, Stephan Windecker, Paul Mohacsi, Lorenz Räber, Vilborg Sigurdardottir.

References

1. Lund LH, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Goldfarb S, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-second Official Adult Heart Transplantation Report—2015; Focus Theme: Early Graft Failure. *J Heart Lung Transplant*. Elsevier; 2015; 34: 1244–1254. <https://doi.org/10.1016/j.healun.2015.08.003> PMID: 26454738
2. Rahmani M, Cruz RP, Granville DJ, McManus BM. Allograft vasculopathy versus atherosclerosis. *Circ Res*. 2006; 99: 801–15. <https://doi.org/10.1161/01.RES.0000246086.93555.f3> PMID: 17038650
3. Clerkin KJ, Ali ZA, Mancini DM. New developments for the detection and treatment of cardiac vasculopathy. *Curr Opin Cardiol*. 2017; 32: 316–325. <https://doi.org/10.1097/HCO.0000000000000388> PMID: 28212149
4. Ruygrok PN, Webber B, Faddy S, Muller DW, Keogh A. Angiographic regression of cardiac allograft vasculopathy after introducing sirolimus immunosuppression. *J Heart Lung Transplant*. 2003; 22: 1276–9. [https://doi.org/10.1016/S1053-2498\(02\)01239-1](https://doi.org/10.1016/S1053-2498(02)01239-1) PMID: 14585389
5. Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S, et al. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant*. Elsevier Inc.; 2010; 29: 914–56. <https://doi.org/10.1016/j.healun.2010.05.034> PMID: 20643330
6. Mancini D, Pinney S, Burkhoof D, Lamanca J, Itescu S, Burke E, et al. Use of Rapamycin Slows Progression of Cardiac Transplantation Vasculopathy. 2003; 4–10. <https://doi.org/10.1161/01.CIR.0000070421.38604.2B> PMID: 12742978
7. Arora S, Andreassen AK, Andersson B, Gustafsson F, Eiskjaer H, Bøtker HE, et al. The Effect of Everolimus Initiation and Calcineurin Inhibitor Elimination on Cardiac Allograft Vasculopathy in De Novo Recipients: One-Year Results of a Scandinavian Randomized Trial. *Am J Transplant*. 2015; 15: 1967–75. <https://doi.org/10.1111/ajt.13214> PMID: 25783974
8. Pollack A, Nazif T, Mancini D, Weisz G. Detection and imaging of cardiac allograft vasculopathy. *JACC Cardiovasc Imaging*. Elsevier Inc.; 2013; 6: 613–23. <https://doi.org/10.1016/j.jcmg.2013.03.001> PMID: 23680373
9. Yeung AC, Davis SF, Hauptman PJ, Kobashigawa JA, Miller LW, Valentine HA, et al. Incidence and progression of transplant coronary artery disease over 1 year: results of a multicenter trial with use of intravascular ultrasound. Multicenter Intravascular Ultrasound Transplant Study Group. *J Heart Lung Transplant*. 1995; 14: S215–20. Available: <http://www.ncbi.nlm.nih.gov/pubmed/8719489> PMID: 8719489
10. Tuzcu EM, Kapadia SR, Sachar R, Ziada KM, Crowe TD, Feng J, 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*. 2005; 45: 1538–42. <https://doi.org/10.1016/j.jacc.2004.12.076> PMID: 15862431
11. Taniwaki M, Windecker S, Zaugg S, Stefanini GG, Baumgartner S, Zanchin T, et al. The association between in-stent neoatherosclerosis and native coronary artery disease progression: a long-term angiographic and optical coherence tomography cohort study. 2015; 2167–2176. <https://doi.org/10.1093/eurheartj/ehv227> PMID: 26040806

12. Reiber JHC, Tu S, Tuinenburg JC, Koning G, Janssen JP, Dijkstra J. QCA, IVUS and OCT in interventional cardiology in 2011. *Cardiovasc Diagn Ther.* 2011; 1: 57–70. <https://doi.org/10.3978/j.issn.2223-3652.2011.09.03> PMID: 24282685
13. Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant.* 2005; 24: 1710–20. <https://doi.org/10.1016/j.healun.2005.03.019> PMID: 16297770
14. Kobashigawa JA, Tobis JM, Starling RC, Tuzcu EM, Smith AL, Valentine HA, et al. Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years. *J Am Coll Cardiol.* 2005; 45: 1532–7. <https://doi.org/10.1016/j.jacc.2005.02.035> PMID: 15862430
15. Wong C, Ganz P, Miller L, Kobashigawa J, Schwarzkopf A, Valentine von Kaepfer H, et al. Role of vascular remodeling in the pathogenesis of early transplant coronary artery disease: a multicenter prospective intravascular ultrasound study. *J Heart Lung Transplant.* 2001; 20: 385–92. PMID: 11295575
16. Tsutsui H, Schoenhagen P, Ziada KM, Crowe TD, Klingensmith JD, Vince DG, et al. Early constriction or expansion of the external elastic membrane area determines the late remodeling response and cumulative lumen loss in transplant vasculopathy: an intravascular ultrasound study with 4-year follow-up. *J Heart Lung Transplant.* 2003; 22: 519–25. [https://doi.org/10.1016/S1053-2498\(02\)01228-7](https://doi.org/10.1016/S1053-2498(02)01228-7) PMID: 12742413
17. Billingham ME. Histopathology of graft coronary disease. *J Heart Lung Transplant.* 1992; 11: S38–44. Available: <http://www.ncbi.nlm.nih.gov/pubmed/1622997> PMID: 1622997
18. Johnson DE, Gao SZ, Schroeder JS, DeCampi WM, Billingham ME. The spectrum of coronary artery pathologic findings in human cardiac allografts. *J Heart Transplant.* 8: 349–59. Available: <http://www.ncbi.nlm.nih.gov/pubmed/2795279> PMID: 2795279
19. Lin H, Wilson JE, Kendall TJ, Radio SJ, Cornhill FJ, Herderick E, et al. Comparable proximal and distal severity of intimal thickening and size of epicardial coronary arteries in transplant arteriopathy of human cardiac allografts. *J Heart Lung Transplant.* 13: 824–33. Available: <http://www.ncbi.nlm.nih.gov/pubmed/7803423> PMID: 7803423
20. Stehlik J, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report—2012. *J Heart Lung Transplant.* Elsevier Inc.; 2012; 31: 1052–64. <https://doi.org/10.1016/j.healun.2012.08.002> PMID: 22975095
21. Delgado JF, Reyne AG, de Dios S, López-Medrano F, Jurado A, Juan RS, et al. Influence of cytomegalovirus infection in the development of cardiac allograft vasculopathy after heart transplantation. *J Heart Lung Transplant.* Elsevier; 2015; 34: 1112–9. <https://doi.org/10.1016/j.healun.2015.03.015> PMID: 25940077
22. Johansson I, Andersson R, Friman V, Selimovic N, Hanzen L, Nasic S, et al. Cytomegalovirus infection and disease reduce 10-year cardiac allograft vasculopathy-free survival in heart transplant recipients. *BMC Infect Dis.* BMC Infectious Diseases; 2015; 15: 582. <https://doi.org/10.1186/s12879-015-1321-1> PMID: 26703239
23. Braga JR, Santos ISO, McDonald M, Shah PS, Ross HJ. Factors associated with the development of cardiac allograft vasculopathy—a systematic review of observational studies. *Clin Transplant.* 2012; 26: E111–24. <https://doi.org/10.1111/j.1399-0012.2011.01565.x> PMID: 22168269