OPEN

Analysis of Fibrotic Plaques in Angiographic Manifest Cardiac Allograft Vasculopathy in Long-term Heart Transplanted Patients Using Optical Coherence Tomography

Madeleine Orban, MD,[1,4] Dominic Dischl, MD,[1,4] Christoph Müller, MD,[2] Sarah Ulrich, MD,[3] Tobias Petzold, MD,[1,4] Konstantinos Rizas, MD,[1,4] Martin W. Orban, MD,[1] Daniel Braun, MD,[1] Jörg Hausleiter, MD,[1,4] Christian Hagl, MD,[2,4] Julinda Mehilli, MD,[1,4] and Steffen Massberg, MD[1,4]

Background. The development and progression of cardiac allograft vasculopathy documented by coronary angiography (CAV_{angio}) after heart transplantation (HTx) has prognostic relevance. Yet there are limited data regarding the role of concomitant intracoronary imaging in the presence CAV_{angio}. In particular, atherosclerotic plaques might represent a potential target for prevention, but their impact on stenosis is understudied. **Methods.** We used high-resolution intracoronary optical coherence tomography (OCT) to quantify and compare findings of intimal hyperplasia (IH) and plaque morphologies in HTx patients (fibrotic plaque, lipid plaque, and calcified plaque). OCT findings were related to the presence of CAV_{angio} as well as to the severity of stenosis. **Results.** We included 65 consecutive patients into analysis (66% with CAV_{angio}, posttransplant interval 9.9 ± 7.6 y). Fibrotic, lipid, and calcified plaques were present in 41 (63.1%), 39 (60%), and 18 (27.7%) patients, respectively. In addition to IH, the presence of fibrotic, lipid, and calcified plaques was found to be associated with CAV_{angio}. The prevalence of lipid plaque and quantitative measurements of fibrotic plaque increased with stenosis severity (lipid plaque, $P < 0.001$, maximal and mean fibrotic arc, $P = 0.05$ and $P = 0.001$, respectively). Receiver operating characteristic analysis showed that area under the curve of the fibrotic plaque parameter mean fibrotic arc (0.87, 95% confidence interval [0.76-0.99]; $P = 0.002$) was superior to area under the curve of intima parameters regarding CAV_{angio}. The effect of mean fibrotic arc ($r = 0.52$, $P < 0.001$) was relevant regarding stenosis severity. **Conclusions.** After a longer posttransplant interval, CAV findings in OCT included a combination of IH and atherosclerotic plaques. In addition to IH, the presence of fibrotic, lipid, and calcified plaques is associated with CAV_{angio}. Further studies are warranted to evaluate if the in vivo screening for plaque progress, particularly of fibrotic plaque, could improve individual secondary prevention and outcome in HTx patients.

(*Transplantation Direct* 2022;8: e1266; doi: 10.1097/TXD.0000000000001266).

Received 19 July 2021. Revision received 24 October 2021.

Accepted 26 October 2021.

[1] Department of Medicine I, University Hospital, LMU Munich, Germany.

[2] Department of Heart Surgery, University Hospital, LMU Munich, Germany.

[3] Department of Paediatric Cardiology and Intensive Care Medicine, University Hospital, LMU Munich, Germany.

[4] German Centre for Cardiovascular Research (DZHK), Partner Site Munich Heart Alliance, Berlin, Germany.

S.U. reports grants from Novartis Pharma GmbH and Astellas Pharma GmbH, outside the submitted work. M.W.O. received speaker honoraria from Abbott Medical, AstraZeneca, Abiomed, Bayer vital, BIOTRONIK, Bristol Myers Squibb, CytoSorbents, Daiichi Sankyo Deutschland, Edwards Lifesciences Services, Sedana Medical, outside the submitted work. D.B. reports speaker honoraria from Abbott Vascular, outside the submitted work. J.H. reports grants and personal fees from Abbott Vascular, grants and personal fees from Edwards Lifesciences, outside the submitted work. J.M. reports grants and personal fees from Boston Scientifics, Astra Zeneca, Bristol Myers Squibb, Edward Life Science, outside the submitted work. S.M. reports grants from German Federal Ministry of Education and Research (BMBF)/German Centre for Cardiovascular Research (DZHK), grants from German Research Foundation (DFG), grants from Boston scientific, grants from Foundation Leduq Transatlantic Network of Excellence, outside the submitted work. The other authors declare no conflicts of interest.

J.M. reports funding of German Centre for Cardiovascular Research (DZHK) for material costs of optical coherence tomography and image analysis.

M.O. and D.D. participated in the data collection. M.O. and S.M. participated in the writing of the article, analytic tools, and research design. S.U., C.M., T.P., K.R., M.W.O., D.B., J.H., C.H., J.M., and S.M. participated in the performance of the research.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantationdirect.com).

Correspondence: Madeleine Orban, MD, Department of Medicine I, University Hospital, LMU Munich, Marchioninistraße 15, 81377 Munich, Germany. (madeleine.orban@med.uni-muenchen.de).

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001266.

Cardiac allograft vasculopathy (CAV) still represents a major limitation to survival after heart transplantation (HTx).[1] Its current definition encompasses angiographic changes (CAV_{angio}), where mortality is already associated with mild angiographic abnormalities and increases with higher stenosis grades.[2,3] Angiography, therefore, represents the gold standard for the detection of CAV. Yet the understanding of CAV_{angio} development and progression is incomplete, leading to a gap of knowledge regarding optimal individual prevention and treatment strategies.

Previous studies with intravascular ultrasound (IVUS) performed in patients with short posttransplant interval suggest that intimal hyperplasia (CAV^{IH}) might be the precursor of CAV_{angio} followed by progressive pathological vascular remodeling.[4,5] Recent studies described not only the presence of absolute intimal hyperplasia (absolute CAV^{IH}) but also of relative intimal hyperplasia defined as abnormal intima-media ratio (I/M ratio, relative CAV^{IH}) in CAV_{angio}.[6-8] An I/M ratio >1 has been defined as abnormal, but whether other cut-off values are more clinically and prognostically relevant is unknown.[9] The advantage of I/M ratio over absolute CAV^{IH} measurements is that it is independent from vessel caliber and segment in which analysis is performed, thereby improving CAV^{IH} detection.[9]

To what extent further pathologic vascular patterns impact on CAV_{angio} is not clear. However, it is fundamental to improve the understanding of pathologic vascular patterns leading to CAV_{angio} to better define primary and secondary prevention strategies. The pathomechanisms and concurrent pathologic vascular patterns of CAV differ markedly depending on the posttransplant interval.[10,11] A high prevalence of atherosclerotic plaques, particularly of lipid, fibrotic, and calcified morphology, has been described in patients with longer posttransplant interval in histological and intracoronary imaging studies, including patients with and without CAV_{angio}.[6,12,13] The clinical and prognostic relevance of atherosclerotic plaques in CAV_{angio} is not established. Yet results suggest that combining findings in angiography and high-resolution intracoronary imaging might help tailor individual therapy, particularly in the less understood cohort of mid- to long-term HTx patients.[8,14]

Because of its high resolution, intracoronary optical coherence tomography (OCT) is a promising tool to evaluate the association of CAV_{angio} with vascular pathologies. Particularly, it offers improved intima and media differentiation and plaque characterization as compared to IVUS, as well as an excellent correlation with histopathology.[6,15,16] Using OCT and novel methods for intima quantification in the presence of plaques,[17] the aim of our study was to evaluate the association of OCT findings with CAV_{angio} at the time point of invasive follow-up in a cohort of mid- to long-term transplanted patients. In this cross-sectional study, we (1) analyzed the association of CAV_{angio} with absolute and relative CAV^{IH}, as well as with fibrotic, lipid, and calcified plaques and (2) related OCT findings to the severity of stenosis in vessels with CAV_{angio}.

MATERIALS AND METHODS

Study Population

We retrospectively analyzed adult HTx patients that received angiography and OCT examinations, performed in our center between July 2016 and January 2021. The study was approved by the local ethics committee (protocol number GEMucI001; IRB number 162-26) and performed in accordance with the ethical standards and the Declaration of Helsinki. All patients gave written consent.

Key Inclusion/Exclusion Criteria

Adult patients that had undergone HTx and had been examined with both coronary angiography and OCT as part of posttransplant follow-up were included into this analysis. The time point of invasive follow-up was defined by our posttransplant care clinic based on the current recommendations.[18,19] Patients with unstable renal function or reasons for potential worsening of renal function due to additional contrast as part of OCT underwent only angiography and are not included into this study. Vessels previously treated with percutaneous coronary intervention or inability to perform sufficient intima and plaques measurements were excluded from the analysis. If patients had undergone multiple invasive follow-up including angiography and OCT during this period, only one OCT examination per patient was chosen for this analysis based on the inclusion and exclusion criteria and the quality for analysis.

Definitions of CAV_{angio} and Severity of Vascular Narrowing

Angiographic manifest CAV (CAV_{angio}) was defined at vessel level and at patient level. CAV_{angio} at vessel level was defined according to the classification of the International Society for Heart and Lung Transplantation (ISHLT) as ISHLT CAV grade >0 in the vessel OCT was performed. Overall CAV_{angio} represents CAV_{angio} at patient level and was defined according to the ISHLT CAV nomenclature.[2] Severity of coronary stenosis was assessed by standard definitions, comprising categories of nondetectable, nonsevere (<70% stenosis), and severe stenosis (≥70% stenosis) according to the standard classification of the ISHLT in primary vessels.[20,21]

OCT Acquisition and Analysis

Images were acquired using an OCT catheter (FastView Coronary Imaging Catheter, Terumo Corporation, Tokyo, Japan) and imaging console (Lunawave system, Terumo Corporation, Tokyo. Japan). The examination was preceded by anticoagulation with 5000 IU heparin. After the intubation of the coronary ostium with a 6 French side-hole guiding catheter, the OCT catheter was placed into the coronary of interest under fluoroscopy control, followed by simultaneous coronary angiography and automatic pull-back of the OCT catheter with image acquisition. Analysis of OCT sequences was performed using validated software (QIvus Medis Program Version 2.5.18, Leiden, The Netherlands).

Definition and Quantification of CAV^{IH}

Quantification of IT and media thickness was performed using a distance measuring method per quadrant, as previously described.[17] As the high OCT resolution allows an excellent intima-media differentiation and offers the possibility of concise intima measurements that correlate with histopathological findings, we defined a maximal IT >0.3 mm as absolute CAV^{IH} and an I/M >1 as abnormal (relative CAV^{IH}) in accordance with the definitions used in HTx patients in previous OCT and histopathological studies.[6,9,17] Prevalence of higher I/M ratios of >2 and >3 was also tested.

Definition and Quantification of Plaques

Plaques were classified as lipid plaques, calcified plaques, or fibrotic plaques according to standard definitions of intravascular optical coherence tomography studies.[22,23] All plaque measurements were performed at 1-mm intervals.[8] Plaques with a length <1 mm were excluded from the analysis. The absolute plaque length and the relative plaque length (in %, defined as percentage of the plaque length compared with the overall length of the vessel analyzed in OCT) were evaluated. As previously described, plaque analysis included delineating lateral plaque borders and thereby measuring the arc of each plaque per frame. An example of arc measurement of fibrotic plaque is represented in Figure 1.[8] For each patient and each plaque morphology, the maximal arc measurement was assessed, and mean arc was calculated based on the measurements performed at 1-mm intervals.

Statistics

Continuous data are expressed as mean (±SD) or median with interquartile range. Normality of data was tested with the Shapiro–Wilk test. Variables were compared with the unpaired Student *t* test/ANOVA test, or Mann–Whitney *U* test/Kruskal–Wallis test, as appropriate. Categorical data are expressed as numbers and percentages and compared with the χ^2 test. Mean values of OCT findings were shown to represent overall disease per vessel, and maximal values were shown to represent the maximal disease per vessel. The association between CAV_{angio} at vessel and at patient level with the prevalence of OCT findings was assessed using odds ratios (OR) and 95% confidence intervals (CIs) for dichotomous variables. The association between the continuous variable of severity of maximum stenosis and continuous parameters from OCT analysis was evaluated using bivariate correlation.[24] Receiver operating characteristic (ROC) curve analysis was performed to assess specificity and sensitivity of continuous OCT parameters regarding the presence of CAV_{angio}. Youden index analysis was used to determine cutoff values of parameters for dichotomization regarding CAV_{angio}. A $P < 0.05$ was considered significant. IBM SPSS statistics version 26 was used for analysis.

RESULTS

Overall Population

Between July 2016 and January 2021, 118 HTx patients received invasive coronary angiography. Eighty-two patients underwent invasive follow-up including a combination of angiography and intracoronary imaging using OCT. Based on inclusion and exclusion criteria, 65 patients were included into this analysis. In the study cohort, age at OCT was 56.8 ± 12.5 y,

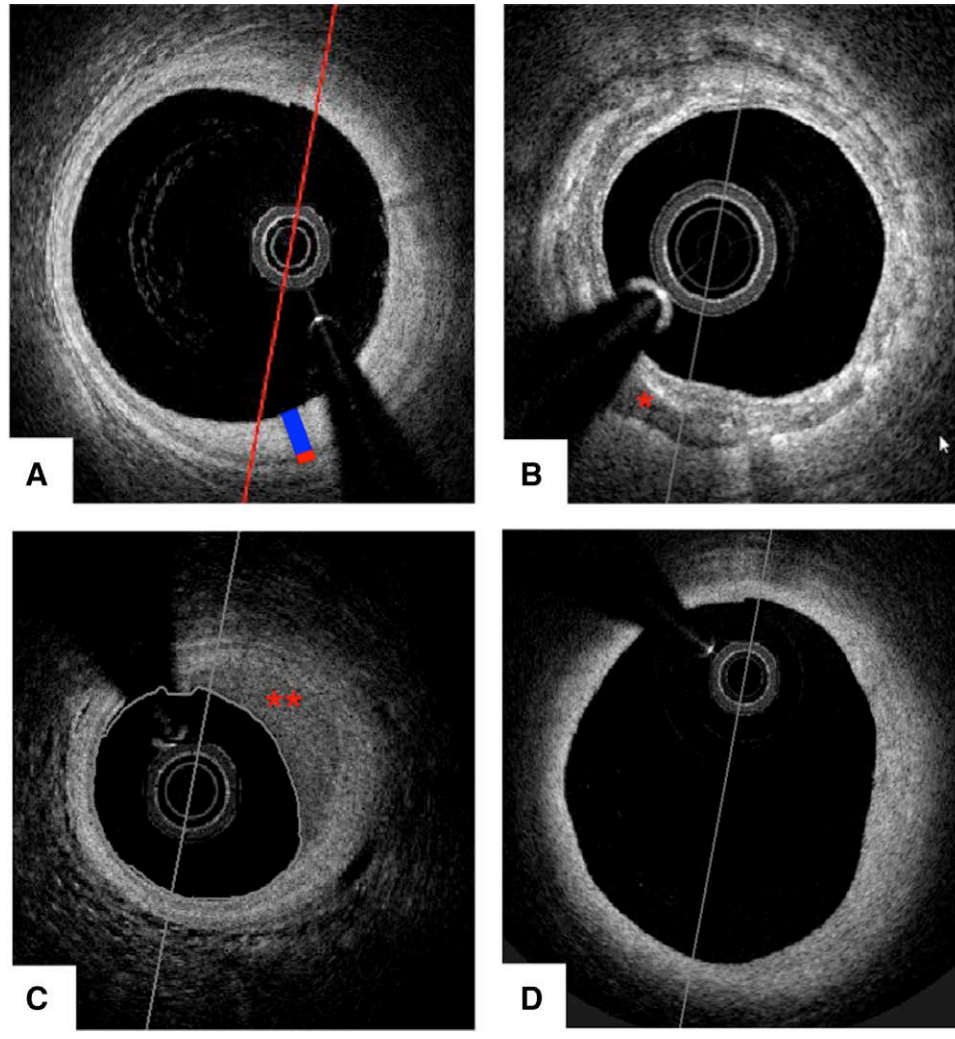

FIGURE 1. Representative optical coherence tomography (OCT) findings. A, Example of eccentric intimal hyperplasia. The blue line represents the intima measurement; the red line represents the media measurement in one quadrant. B, Circular calcified plaque. C, Lipid plaque. D, Fibrotic plaque. Border between lateral plaque borders and normal vessel wall are delineated and thereby the arc of the plaque is measured.

and 54 patients (83.1%) were male. The posttransplant interval was 9.9 ± 7.6 y. CAV_{angio} was found in 43 patients (66.2%). Fibrotic, lipid, and calcified plaques were present in 41 (63.1%), 39 (60%), and 18 (27.7%) patients, respectively. Characteristics of the overall study population are shown in Table 1, and representative images of OCT findings are shown in Figure 1.

OCT Findings in Patients With Versus Without Angiographic Changes Due to CAV (CAV_{angio})

All patients presented an I/M >1, and patients without and with CAV_{angio} had a similar prevalence of maximal I/M >2 (86.4% versus 90.2%, P = 0.6). Figure 2 shows differences regarding the prevalence of OCT findings in patients with versus without CAV_{angio}. The prevalence of absolute intimal hyperplasia (absolute CAV^{IH}) with IT >0.3 mm and relative CAV^{IH} with I/M >3 was significantly higher in the cohort with CAV_{angio} compared with patients without CAV_{angio} (absolute CAV^{IH}: 40.9% versus 75.6%, P = 0.006; relative CAV^{IH} with I/M >3: 45.5% versus 78.1%, P = 0.009). Also, the prevalence of fibrotic, lipid, and calcified plaques was significantly higher in the cohort with CAV_{angio} (fibrotic: 31.8% versus 83.7%, P < 0.001; lipid: 13.6% versus 93.0%, P < 0.001; calcified: 4.5% versus 53.5%, P = 0.003).

Association of OCT Findings With CAV_{angio} per Vessel

Table 2 shows the association between dichotomous OCT findings and CAV_{angio}. The OR (95% CI) for association of absolute CAV^{IH} (IT >0.3 mm) with CAV_{angio} was 4.48 (1.48-13.58). The OR of relative CAV^{IH} was 1.46 (0.30-7.20) if defined by I/M >2, and 4.27 (1.39-13.06) if defined as I/M >3. The OR for the association with CAV_{angio} was 8.10 (2.54-25.81) for fibrotic plaques, 32.57 (7.55-140.57) for lipid plaques, and 13.73 (1.69-111.81) for calcified plaques.

TABLE 1.

Population characteristics

Characteristics (n = 65)	
Male sex, n (%)	54 (83.1)
Age at OCT, y	56.8 ± 12.5
Posttransplant interval, y	9.9 ± 7.6
CAV_{angio},[a] n (%)	43 (66.2)
Severity of angiographic stenosis in the analyzed vessel[a]	
Nondetectable stenosis, n (%)	22 (33.8)
Nonsevere stenosis, n (%)	31 (47.7)
Severe stenosis, n (%)	12 (18.5)
ISHLT CAV grade	
ISHLT CAV grade 0, n (%)	20 (30.8)
ISHLT CAV grade 1, n (%)	30 (46.2)
ISHLT CAV grade 2 and 3,[b] n (%)	15 (23.1)
Reason for index HTx	
Ischemic cardiomyopathy, n (%)	17 (26.2)
Dilated cardiomyopathy, n (%)	30 (46.2)
Others,[c] n (%)	18 (27.7)
Medication at OCT	
Prednisolone, n (%)	4 (6.2)
Tacrolimus, n (%)	51 (78.5)
Cyclosporine, n (%)	5 (7.7)
Everolimus, n (%)	8 (12.3)
Sirolimus, n (%)	16 (24.6)
Mycophenolate, n (%)	50 (76.9)
Statin, n (%)	54 (83.1)

Data are shown as mean ± SD or n (%).

[a]CAV_{angio} and severity of angiographic stenosis were defined according to the standard classification of the ISHLT in primary vessels.

[b]Includes patients having undergone PCI in another vessel due to high-grade CAV (ISHLT CAV grades 2 and 3).

[c]Others include myocarditis, peripartal cardiomyopathy, retransplantation, and restrictive cardiomyopathy.

CAV_{angio}, angiographic manifest cardiac allograft vasculopathy; HTx, heart transplantation; ISHLT, International Society for Heart and Lung Transplantation; OCT, optical coherence tomography; PCI, percutaneous coronary intervention.

OCT Findings According to Severity of Stenosis per Vessel

Twenty-two (33.9%) patients had no detectable angiographic change, 31 (47.7%) patients had nonsevere angiographic stenosis, and 12 (18.5%) patients had severe angiographic stenosis in the analyzed vessel. Table 3 shows the qualitative and quantitative OCT measurements of intima and each plaque morphology according to stenosis category. The prevalence of absolute and relative CAV^{IH} was lower in patients without stenosis compared with patients with nonsevere stenosis (P = 0.02). The prevalence of fibrotic, lipid, and calcified plaques increased significantly with higher stenosis severity (P = 0.001, P < 0.001, and P = 0.007, respectively). With increasing stenosis severity, there was a significant quantitative increase of fibrotic plaque measurements (maximal and mean fibrotic arc, P = 0.05 and P = 0.001, respectively).

Effect of Continuous OCT Measurements Regarding Severity of Stenosis per Vessel

The correlation of OCT findings with severity of angiographic stenosis is shown in Table 4. A significant positive correlation between absolute and relative measurements of intima and of fibrotic plaques parameters with the severity of angiographic stenosis was found. The strongest effect was seen in mean fibrotic arc (r = 0.52, P < 0.001) regarding the severity of angiographic stenosis, as compared to other quantitative OCT parameters: maximal IT (r = 0.39, P = 0.002), mean IT (r = 0.43, P < 0.001), maximal I/M (r = 0.32, P = 0.01), mean I/M (r = 0.39, P = 0.001), relative length of fibrotic plaque (r = 0.32, P = 0.04), maximal fibrotic arc (r = 0.33, P = 0.03), and relative length of lipid plaque (r = 0.40, P = 0.01).

ROC Analyses of Continuous OCT Findings Regarding the Presence of CAV_{angio} and Assessment of Cutoff Values

The ROC analysis is presented in Figure 3. ROC curve showed the highest area under the curve for mean fibrotic arc (0.87, 95% CI [0.76-0.99]; P = 0.002), as compared with absolute and relative CAV^{IH} (maximal IT: 0.77 [0.65-0.89], P < 0.001; mean IT: 0.75 [0.63-0.87], P = 0.001; maximal I/M: 0.75 [0.63-0.87], P = 0.001; mean I/M: 0.76 [0.64-0.87], P = 0.001). Area under the curve of other OCT parameters did not differ significantly from 0.5.

We determined optimal cutoff values for mean fibrotic arc as well as for IT and I/M by Youden index for the association with CAV_{angio}. Mean fibrotic arc and maximal IT had high specificity regarding CAV_{angio}: mean fibrotic arc ≥240 degree (specificity = 100%, sensitivity = 65%) and maximal IT ≥0.31 mm (specificity = 100%, sensitivity = 76%). Using a cutoff value for maximal I/M of ≥5.48, specificity was also excellent (specificity = 100%, sensitivity = 44%) and medium if using ≥3.49 (specificity = 68%, sensitivity = 76%). Mean I/M showed also an excellent specificity using the cutoff value ≥2.14 (specificity = 96%, sensitivity = 59%). Specificity and

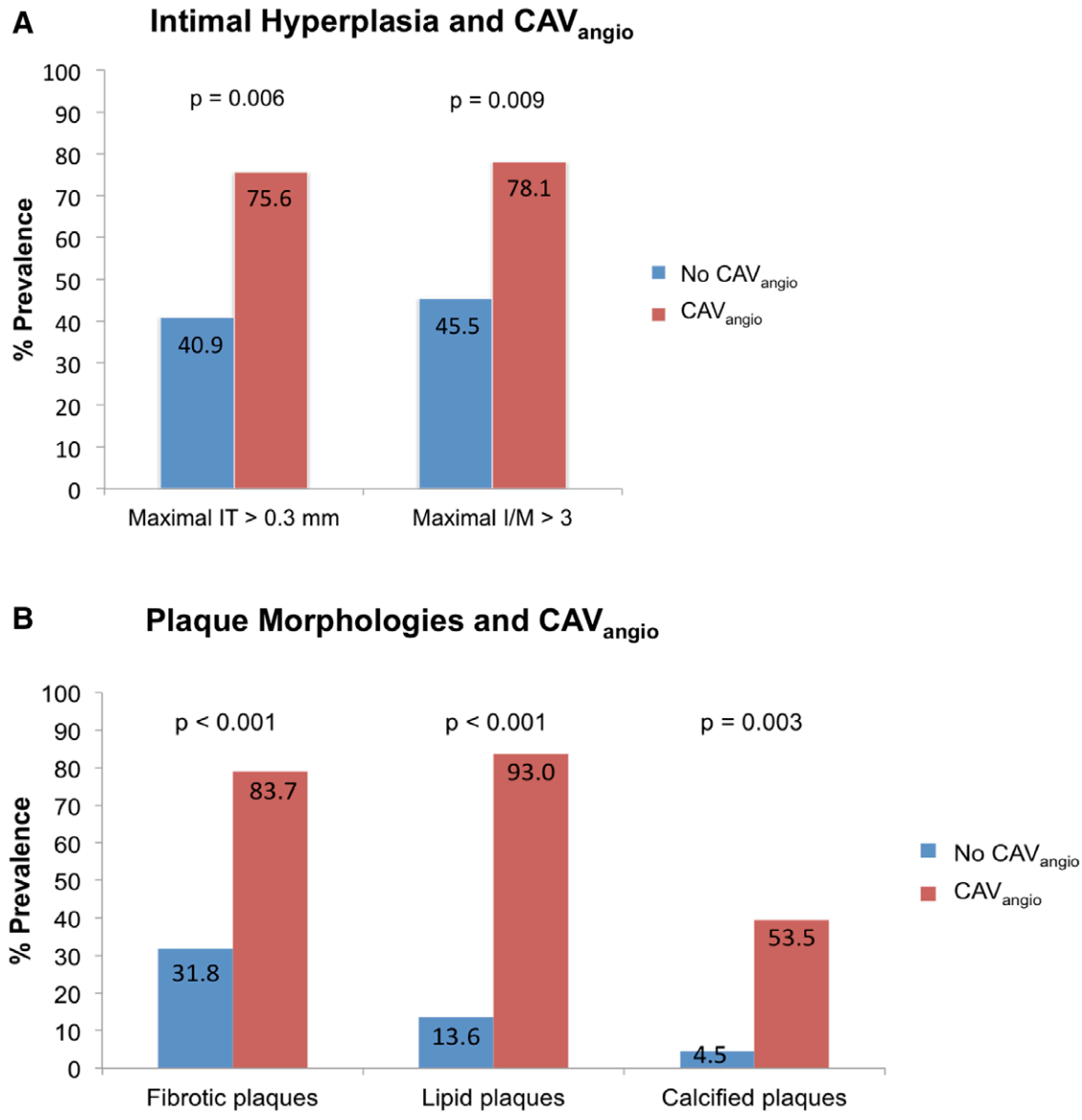

FIGURE 2. Optical coherence tomography (OCT) findings in patients with and without angiographic manifest cardiac allograft vasculopathy (CAV_{angio}). Patients without CAV_{angio}, n = 22. Patients with CAV_{angio}, n = 43. A, Prevalence of absolute and relative CAV^{IH}. Maximal intimal thickness (IT) >0.3 mm denotes absolute CAV^{IH}. Maximal intima-media ratio (I/M) >3 denotes relative CAV^{IH} with a cutoff of I/M >3. B, Prevalence of fibrotic, lipid, and calcified plaques. CAV_{angio} was defined according to the classification of the International Society for Heart and Lung Transplantation (ISHLT) as ISHLT CAV grade >0 in the analyzed vessel. *P* values are shown for χ^2 test.

TABLE 2.

Association of OCT findings with CAV_{angio} at vessel level

Factor	Odds ratio (95% CI)
Intimal hyperplasia	
Absolute intimal hyperplasia	4.48 (1.48-13.58)
Relative intimal hyperplasia with I/M >2	1.46 (0.30-7.20)
Relative intimal hyperplasia with I/M >3	4.27 (1.39-13.06)
Plaques morphologies	
Fibrotic plaques	8.10 (2.54-25.81)
Calcified plaques	13.73 (1.69-111.81)
Lipid plaques	32.57 (7.55-140.57)

CAV_{angio}, angiographic manifest cardiac allograft vasculopathy; CI, confidence interval; I/M, intima-media ratio; OCT, optical coherence tomography.

sensitivity were medium for mean IT using the cutoff value ≥0.18 mm (specificity = 77%, sensitivity = 61%).

Association of OCT Findings With Overall CAV_{angio} at Patient Level (According to ISHLT CAV Classification)

Table 5 shows the association of OCT findings with overall CAV_{angio}. Fibrotic and lipid plaques were associated with ISHLT CAV 1 (3.10 [1.06-9.09] and 3.90 [1.33-11.45], respectively). Lipid plaques were associated with ISHLT CAV 2/3 (6.00 [1.23-29.39]). A borderline association with ISHLT CAV 1 was found for I/M >3 (3.03 [0.98-9.32]).

Effect of Cardiovascular Risk Factors

With higher severity of stenosis, there was a higher prevalence of diabetes (*P* = 0.004, **Table S1, SDC**, http://links.lww.com/TXD/A393). Arterial hypertension was associated with fibrotic and calcified plaques in our cohort (**Table S2, SDC**, http://links.lww.com/TXD/A393).

TABLE 3.

OCT findings according to angiographic stenosis severity at vessel level

	Nondetectable stenosis, n = 22	Nonsevere stenosis, n = 31	Severe stenosis, n = 12	*P*
Intimal hyperplasia				
Patients with maximal IT >0.3 mm, n (%)	9 (40.9)	23 (74.2)	8 (66.7)	0.02[a]
Patients with maximal I/M >3	8 (36.4)	24 (77.4)	8 (66.7)	0.03[a]
Maximal IT, mm	0.3 ± 0.1	0.5 ± 0.4	0.5 ± 0.3	0.006[a]
Mean IT, mm	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.002[a]
Maximal I/M	3.1 ± 1.1	6.2 ± 3.9	5.4 ± 4.1	0.004[a]
Mean I/M	1.6 ± 0.5	2.5 ± 1.2	2.4 ± 1.3	0.004[a]
Fibrotic plaque				
Patients with fibrotic plaque, n (%)	7 (31.8)	24 (77.4)	10 (83.3)	0.001[a]
Relative plaque length, %	55.0 ± 44.4	72.4 ± 26.5	77.9 ± 24.3	0.045
Maximal fibrotic arc, deg	306.6 ± 57.1	343.6 ± 36.1	360.0 ± 0.0	0.05[a]
Mean fibrotic arc, deg	215.1 ± 14.3	250.0 ± 42.7	289.8 ± 46.0	0.001[a]
Lipid plaque				
Patients with lipid plaque, n (%)	3 (13.6)	25 (80.6)	11 (91.7)	<0.001[a]
Relative plaque length, %	18.6 ± 9.8	40.6 ± 34.9	56.1 ± 30.5	0.2
Maximal lipid arc, deg	167.9 ± 53.3	205.8 ± 89.8	241.0 ± 68.9	0.3
Mean lipid arc, deg	127.1 ± 28.9	123.8 ± 43.7	139.3 ± 40.7	0.9
Calcified plaque				
Patients with calcified plaque, n (%)	1 (4.5)	11 (35.5)	6 (50.0)	0.007[a]
Relative plaque length, %	NA	30.5 ± 25.8	28.0 ± 29.5	0.6
Maximal calcified arc, deg	NA	184.5 ± 87.4	186.8 ± 106.5	0.06
Mean calcified arc, deg	NA	104.6 ± 87.4	144.1 ± 98.8	0.38

[a]indicates significant differences between groups with $P < 0.05$.
Data are shown as mean (±SD) or n (%). Relative plaque length was defined as percentage of the plaque length in relation to the overall length of the vessel analyzed in OCT.
deg, arc degree; I/M, intima-media ratio; IT, intimal thickness; NA, not available; OCT, optical coherence tomography.

TABLE 4.

Correlation of OCT findings with severity of angiographic stenosis at vessel level

	r	*P*
Intimal hyperplasia		
Maximal IT, mm	0.39	0.002[a]
Mean IT, mm	0.43	<0.001[a]
Maximal I/M	0.32	0.01[a]
Mean I/M	0.39	0.001[a]
Fibrotic plaque		
Relative plaque length, %	0.32	0.04[a]
Maximal fibrotic arc, deg	0.33	0.03[a]
Mean fibrotic arc, deg	0.52	<0.001[a]
Lipid plaque		
Relative plaque length, %	0.40	0.01[a]
Maximal lipid arc, deg	0.32	0.05
Mean lipid arc, deg	0.22	0.2
Calcified plaque		
Relative plaque length, %	0.02	0.9
Maximal calcified arc, deg	−0.2	0.5
Mean calcified arc, deg	−0.09	0.7

[a]indicates significant correlation with with $P < 0.05$.
deg, arc degree; I/M, intima-media ratio; IT, intimal thickness; OCT, optical coherence tomography.

DISCUSSION

The main findings in our cohort of mid- to long-term heart transplanted adults can be summarized as follows: (1) intracoronary findings of CAV included a combination of CAV^{IH} and atherosclerotic plaques; (2) the presence of CAV_{angio} at vessel level was associated with the presence of fibrotic, lipid, and calcified plaques; (3) stenosis severity at vessel level correlated predominantly with the overall size of fibrotic plaques; and (4) fibrotic and lipid plaques were associated with overall CAV_{angio} at patient level, as defined by *ISHLT* CAV nomenclature. Atherosclerotic plaques, particularly of fibrotic morphology, are therefore a characteristic pathologic vascular pattern in HTx patients associated with CAV_{angio}.

Using a novel method optimizing intima quantification in the presence of plaques,[17] our results strengthen the hypothesis that after a longer posttransplant interval, CAV represents a combination of immune-mediated CAV^{IH} and nonimmune mediated atherosclerotic changes.[6,8,9,12-14] Including atherosclerotic changes into CAV definition might help adjust prevention and treatment strategies for CAV in this cohort of HTx patients. Based on studies in recently transplanted patients, the cornerstone of CAV prevention, delayed CAV progression, and potential CAV reversal currently remains the adjustment of immunosuppressive therapy.[18,25] However, the effect of immunosuppression might be limited to CAV^{IH} and not extend to plaques in HTx patients, and these positive effects could be absent in patients with longer posttransplant interval.[26] These gaps of knowledge highlight the relevance of a better understanding of CAV_{angio} development in these patients.

To evaluate the clinical relevance of atherosclerotic plaques, we assessed their effect regarding the prevalence and severity of CAV_{angio} at vessel and at patient level. We compared findings to CAV^{IH}, an established risk factor for CAV_{angio}.

By means of its high resolution, OCT provides an excellent discrimination of intima and media and a high correlation with histological examinations.[27] We, therefore, used the recently introduced definition of IT >0.3 mm in OCT as definition of absolute CAV^{IH} that corresponds to the histopathological definition and excludes a potential impact of media disease.[6,7,12,28-30] Our results in mid- to long-term transplanted patients are in line with previous studies establishing CAV^{IH}

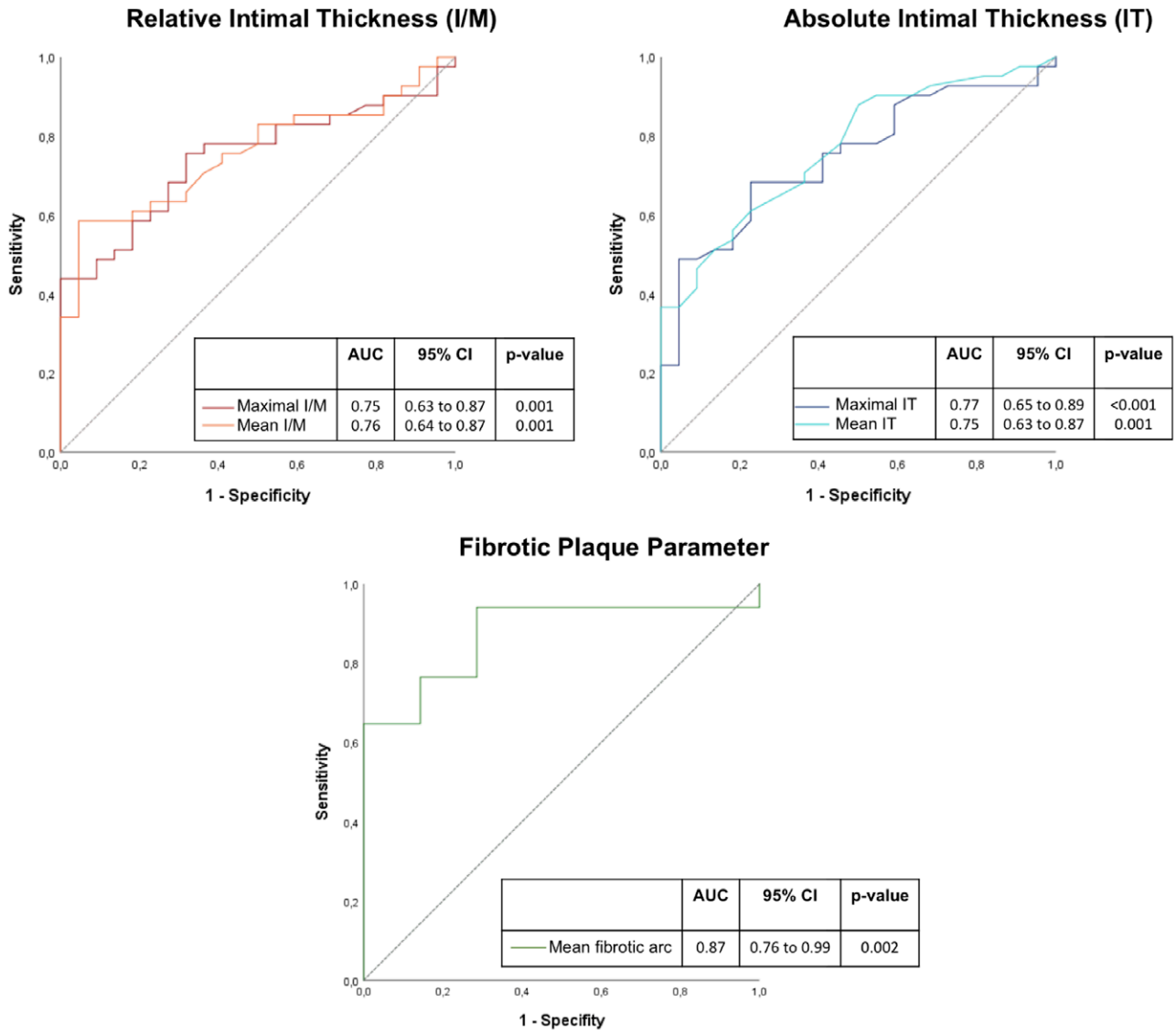

FIGURE 3. Receiver operating characteristic curves of relevant optical coherence tomography (OCT) parameters contributing to the presence of angiographic manifest cardiac allograft vasculopathy (CAV_{angio}). AUC, area under the curve; CI, confidence interval; I/M, intima-media ratio; IT, intimal thickness.

TABLE 5.

Association of OCT findings with overall CAV_{angio} at patient level

Factor	Overall CAV_{angio} ISHLT CAV grade 1	Overall CAV_{angio} ISHLT CAV grades 2 and 3[a]
Intimal hyperplasia		
Absolute intimal hyperplasia	2.07 (0.72-5.98)	2.53 (0.63-10.23)
Relative intimal hyperplasia with I/M >2	1.16 (0.24-5.65)	1.81 (0.20-16.47)
Relative intimal hyperplasia with I/M >3	3.03 (0.98-9.32)	2.13 (0.52-8.66)
Plaques morphologies		
Fibrotic plaques	3.10 (1.06-9.09)[b]	1.83 (0.51-6.57)
Lipid plaques	3.90 (1.33-11.45)[b]	6.00 (1.23-29.39)[b]
Calcified plaques	2.32 (0.76-7.04)	2.11 (0.62-7.15)

[a] includes patients having undergone PCI in another vessel due to high-grade CAV (ISHLT CAV grades 2 and 3).
[b] indicates significant association with $P < 0.05$.
Data are shown as odds ratio (95% CI).
CAV_{angio}, angiographic manifest cardiac allograft vasculopathy; CI, confidence interval; I/M, intima-media ratio; ISHLT, International Society for Heart and Lung Transplantation; OCT, optical coherence tomography; PCI, percutaneous coronary intervention.

as a risk factor for CAV_{angio} after a short posttransplant interval.[4,5] In addition, we assessed the effect of the recent concept of relative CAV^{IH}.[6-8] Our findings add information regarding its clinical relevance, showing that an abnormal I/M >1 was present in >40% of our patients without CAV_{angio}. It is, therefore, unclear if this cutoff represents an early sign of CAV_{angio} development and to what extent this cutoff is suitable to assess CAV_{angio}. The finding that relative CAV^{IH} with maximal I/M >3 is associated with CAV_{angio} reflects the cutoff of I/M >3 found in nontransplanted patients correlating with severe stenosis.[28] Yet the optimal cutoff for follow-up in HTx patients to predict CAV_{angio} might be even higher.

The comparison of our OCT findings suggests a combined or overlapping development of CAV^{IH} and atherosclerotic plaques leading to severe CAV_{angio}. At vessel level, differences of quantitative CAV^{IH} findings were predominantly found between patient cohorts with no stenosis compared with nonsevere stenosis. As opposed, there was a continuous increase of plaques findings with higher stenosis severity, notably of fibrotic plaques parameters. Lipid plaque prevalence also increased significantly according to angiographic stenosis

grade at vessel level. Although the relative length of lipid plaque had a relevant association with stenosis severity, there was only a borderline effect of maximal lipid arc. This might be underestimated due to the number of patients.

To evaluate the association of OCT findings with overall CAV_{angio}, we used the ISHLT CAV nomenclature. The ISHLT CAV nomenclature is recommended for the prognostic evaluation of CAV_{angio} at patient level in routine follow-up, as it correlates severity grades with mortality.[18] It differentiates proximal and distal lesions, with proximal lesions having a higher impact regarding severity grading and survival. At patient level, we found an association of fibrotic and lipid plaques with prognostically relevant, overall CAV_{angio} that highlights their clinical relevance in HTx patients.[2,3] Although fibrotic plaque prevalence was associated with low-grade overall CAV_{angio}, lipid plaque prevalence was also related to high-grade CAV_{angio}.

Our results reflect and extend data from HTx patients that layered fibrotic plaques could be predictive of the combined end point of vessel occlusion, severe new angiographic coronary stenosis, or percutaneous coronary intervention.[8] The effect of fibrotic plaque on CAV_{angio} severity at vessel level is in line with results in nontransplanted patients. Here, vessels with a higher degree of stenosis were pathologically remodeled vessels that consisted predominantly of fibrotic plaque.[31] If the quantitative parameter of mean fibrotic arc, defining the individual overall burden of fibrotic disease in a patient's vessel, can be used as an additional surrogate to assess progress of CAV_{angio} during follow-up needs further prospective analysis. In addition to fibrotic plaques parameters, lipid plaques measurements were also relevant prognostic parameters in nontransplanted patients.[32]

If results from nontransplanted patients regarding development and progress of atherosclerosis can be transferred directly to HTx patients is not known. In nontransplanted patients, atherosclerotic changes have been described to originate from intimal hyperplasia, and results also showed that fibrotic plaques can further develop into other atherosclerotic plaque morphologies related to stenosis, particularly in the presence of cardiovascular risk factors.[33-35]

In our study, there was a higher prevalence of diabetes with increasing stenosis severity. However, diabetes was not associated with a specific plaque morphology. Although diabetes is known to be one of the most potent risk factors for the development and progression of atherosclerosis in nontransplanted patients, the association of diabetes with CAV_{angio} and stenosis severity is still under debate in HTx patients.[36,37] Establishing the effect of diabetes is challenging, possibly because differences in duration of diabetes, glycemic control, and potential interactions with other cardiovascular risk factors.[37,38] The more stringent association of arterial hypertension with atherosclerotic plaques, particularly of calcified morphology, is in accordance with results in nontransplanted patients.[39,40]

If CAV^{IH} could represent a precursor of atherosclerotic changes in the context of a predisposing metabolic environment in HTx patients has not been analzsed so far. Combining previous results regarding intimal hyperplasia and atherosclerotic changes, and the commonly found metabolic disorders in HTx patients, a similar development could be plausible.[11,34,41-43] However, this is a cross-sectional study evaluating the association of atherosclerotic plaques findings in OCT with CAV_{angio} at the time point of invasive follow-up. It is, therefore, hypothesis-generating, encouraging advanced intracoronary imaging studies in HTx patients for in-depth analysis of development and progress of CAV_{angio}. Future longitudinal studies are essential to evaluate this relevant gap of knowledge in HTx patients and to assess to what extent the use of intracoronary imaging in CAV_{angio} might help individualize secondary prevention and therapy based on the predominant pathology. Whether individualizing prevention strategies by adapting immunosuppressive therapy or including prevention of atherosclerosis risk factors can improve survival can only be resolved in accordingly designed prospective studies.

CONCLUSION

In addition to intimal hyperplasia, the presence of fibrotic, lipid, and calcified plaques is a hallmark of prognostically relevant, angiographic CAV in long-term HTx patients. Particularly quantitative measurements of fibrotic plaque were related to the severity of stenosis, suggesting an important role of fibrotic plaques in the development of CAV. Further studies are warranted to evaluate if the in vivo screening for plaque progress might help individualize secondary prevention of angiographic CAV.

LIMITATIONS

This is a retrospective study. We correlated OCT findings with angiographic CAV and stenosis severity at the time point of OCT, but we cannot provide serial OCT measurements that could potentially demonstrate the impact of our OCT findings on progression of angiographic CAV or confirm CAV^{IH} as a precursor of atherosclerotic plaques in HTx patients. The effects of individual immunosuppression or conventional cardiovascular risk factors regarding findings in OCT need to be assessed in prospective studies. Despite the fact that the number of patients in our cohort is comparable to other OCT studies in the vulnerable population of HTx patients, the absolute number of patients is still relatively small. As we assessed the relevance of OCT findings in adults, we cannot extend findings regarding the effects of CAV^{IH} and atherosclerotic plaques to transplanted children.

REFERENCES

1. Chih S, Chong AY, Mielniczuk LM, et al. Allograft vasculopathy: the Achilles' heel of heart transplantation. *J Am Coll Cardiol.* 2016;68:80–91.
2. Mehra MR, Crespo-Leiro MG, Dipchand A, et al. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. *J Heart Lung Transplant.* 2010;29:717–727.
3. Tremblay-Gravel M, Racine N, de Denus S, et al. Changes in outcomes of cardiac allograft vasculopathy over 30 years following heart transplantation. *JACC Heart Fail.* 2017;5:891–901.
4. Pethig K, Klauss V, Heublein B, et al. Progression of cardiac allograft vascular disease as assessed by serial intravascular ultrasound: correlation to immunological and non-immunological risk factors. *Heart.* 2000;84:494–498.
5. Tsutsui H, Ziada KM, Schoenhagen P, et al. Lumen loss in transplant coronary artery disease is a biphasic process involving early intimal thickening and late constrictive remodeling: results from a 5-year serial intravascular ultrasound study. *Circulation.* 2001;104:653–657.
6. Cassar A, Matsuo Y, Herrmann J, et al. Coronary atherosclerosis with vulnerable plaque and complicated lesions in transplant recipients: new insight into cardiac allograft vasculopathy by optical coherence tomography. *Eur Heart J.* 2013;34:2610–2617.
7. McGovern E, Hosking MCK, Balbacid E, et al. Optical coherence tomography for the early detection of coronary vascular changes in children and

adolescents after cardiac transplantation: findings from the International Pediatric OCT Registry. *JACC Cardiovasc Imaging.* 2019;12:2492–2501.
8. Clemmensen TS, Holm NR, Eiskjær H, et al. Layered fibrotic plaques are the predominant component in cardiac allograft vasculopathy: systematic findings and risk stratification by OCT. *JACC Cardiovasc Imaging.* 2017;10:773–784.
9. Khandhar SJ, Yamamoto H, Teuteberg JJ, et al. Optical coherence tomography for characterization of cardiac allograft vasculopathy after heart transplantation (OCTCAV study). *J Heart Lung Transplant.* 2013;32:596–602.
10. Johnson DE, Gao SZ, Schroeder JS, et al. The spectrum of coronary artery pathologic findings in human cardiac allografts. *J Heart Transplant.* 1989;8:349–359.
11. Valantine HA. Cardiac allograft vasculopathy: central role of endothelial injury leading to transplant "atheroma". *Transplantation.* 2003;76:891–899.
12. Lu WH, Palatnik K, Fishbein GA, et al. Diverse morphologic manifestations of cardiac allograft vasculopathy: a pathologic study of 64 allograft hearts. *J Heart Lung Transplant.* 2011;30:1044–1050.
13. McManus BM, Horley KJ, Wilson JE, et al. Prominence of coronary arterial wall lipids in human heart allografts. Implications for pathogenesis of allograft arteriopathy. *Am J Pathol.* 1995;147:293–308.
14. Sarno G, Lerman A, Bae JH, et al. Multicenter assessment of coronary allograft vasculopathy by intravascular ultrasound-derived analysis of plaque composition. *Nat Clin Pract Cardiovasc Med.* 2009;6:61–69.
15. Tissot C-M. Comparison of optical coherence tomography (OCT) and intravascular ultrasound (IVUS) for the early diagnosis of cardiac allograft vasculopathy in heart transplant patients. *J Heart Lung Transplant*. 2013;32:209.
16. Yabushita H, Bouma BE, Houser SL, et al. Characterization of human atherosclerosis by optical coherence tomography. *Circulation.* 2002;106:1640–1645.
17. Orban M, Ulrich S, Dischl D, et al. Cardiac allograft vasculopathy: differences of absolute and relative intimal hyperplasia in children versus adults in optical coherence tomography. *Int J Cardiol.* 2021;328:227–234.
18. Costanzo MR, Dipchand A, Starling R, et al; International Society of Heart and Lung Transplantation Guidelines. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant.* 2010;29:914–956.
19. Badano LP, Miglioranza MH, Edvardsen T, et al; Document reviewers. European Association of Cardiovascular Imaging/Cardiovascular Imaging Department of the Brazilian Society of Cardiology recommendations for the use of cardiac imaging to assess and follow patients after heart transplantation. *Eur Heart J Cardiovasc Imaging.* 2015;16:919–948.
20. Mehra MR, et al. ISHLT Cardiac Allograft Vaculopathy Grading report. *J Heart Lung Transplant*. 2010;29:717–717.
21. Sharples LD, Jackson CH, Parameshwar J, et al. Diagnostic accuracy of coronary angiography and risk factors for post-heart-transplant cardiac allograft vasculopathy. *Transplantation.* 2003;76:679–682.
22. Kini AS, Vengrenyuk Y, Yoshimura T, et al. Fibrous cap thickness by optical coherence tomography in vivo. *J Am Coll Cardiol.* 2017;69:644–657.
23. Tearney GJ, Regar E, Akasaka T, et al; International Working Group for Intravascular Optical Coherence Tomography (IWG-IVOCT). Consensus standards for acquisition, measurement, and reporting of intravascular optical coherence tomography studies: a report from the International Working Group for Intravascular Optical Coherence Tomography Standardization and Validation. *J Am Coll Cardiol.* 2012;59:1058–1072.
24. Schober P, Boer C, Schwarte LA. Correlation coefficients: appropriate use and interpretation. *Anesth Analg.* 2018;126:1763–1768.
25. Eisen HJ, Tuzcu EM, Dorent R, et al; RAD B253 Study Group. Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. *N Engl J Med.* 2003;349:847–858.
26. Arora S, Ueland T, Wennerblom B, et al. Effect of everolimus introduction on cardiac allograft vasculopathy–results of a randomized, multicenter trial. *Transplantation.* 2011;92:235–243.
27. Kume T, Akasaka T, Kawamoto T, et al. Assessment of coronary intima–media thickness by optical coherence tomography: comparison with intravascular ultrasound. *Circ J.* 2005;69:903–907.
28. Velican C, Velican D. Study of coronary intimal thickening. *Atherosclerosis.* 1985;56:331–344.
29. Velican D, Velican C. Comparative study on age-related changes and atherosclerotic involvement of the coronary arteries of male and female subjects up to 40 years of age. *Atherosclerosis.* 1981;38:39–50.
30. Nissen SE, Gurley JC, Grines CL, et al. Intravascular ultrasound assessment of lumen size and wall morphology in normal subjects and patients with coronary artery disease. *Circulation.* 1991;84:1087–1099.
31. Rodriguez-Granillo GA, Serruys PW, Garcia-Garcia HM, et al. Coronary artery remodelling is related to plaque composition. *Heart.* 2006;92:388–391.
32. Xing L, Higuma T, Wang Z, et al. Clinical significance of lipid-rich plaque detected by optical coherence tomography: a 4-year follow-up study. *J Am Coll Cardiol.* 2017;69:2502–2513.
33. Insull W Jr. The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. *Am J Med.* 2009;122:S3–S14.
34. Stary HC, Blankenhorn DH, Chandler AB, et al. A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation.* 1992;85:391–405.
35. Virmani R, Kolodgie FD, Burke AP, et al. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol.* 2000;20:1262–1275.
36. Poznyak A, Grechko AV, Poggio P, et al. The diabetes mellitus-atherosclerosis connection: the role of lipid and glucose metabolism and chronic inflammation. *Int J Mol Sci*. 2020;21:1835.
37. Potena L, Valantine HA. Cardiac allograft vasculopathy and insulin resistance–hope for new therapeutic targets. *Endocrinol Metab Clin North Am.* 2007;36:965–981;ix.
38. Katakami N. Mechanism of development of atherosclerosis and cardiovascular disease in diabetes mellitus. *J Atheroscler Thromb.* 2018;25:27–39.
39. Kalra SS, Shanahan CM. Vascular calcification and hypertension: cause and effect. *Ann Med.* 2012;44:S85–S92.
40. Saleh M, Alfaddagh A, Elajami TK, et al. Diastolic blood pressure predicts coronary plaque volume in patients with coronary artery disease. *Atherosclerosis.* 2018;277:34–41.
41. Valantine H. Cardiac allograft vasculopathy after heart transplantation: risk factors and management. *J Heart Lung Transplant.* 2004;23:S187–S193.
42. Noel A, Genest J. Smashing low-density lipoprotein levels and preventing coronary allograft vasculopathy: one heart transplant patient at a time. *Can J Cardiol.* 2019;35:17–18.
43. Park JW, Merz M, Braun P, et al. Lipid disorder and transplant coronary artery disease in long-term survivors of heart transplantation. *J Heart Lung Transplant.* 1996;15:572–579.