Comparison Between Cardiac Allograft Vasculopathy and Native Coronary Atherosclerosis by Optical Coherence Tomography

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We sought to explore differences in distribution and morphology of coronary lesions comparing cardiac allograft vasculopathy and native coronary atherosclerosis (NCA) using intravascular imaging with optical coherence tomography (OCT). At the time of routine surveillance angiography, 17 heart transplant (HT) recipients with a history of high-grade cellular rejection (HGR) and 43 HT recipients with none/mild (low)-grade rejection underwent OCT imaging of the left anterior descending and were compared to 60 patients with NCA without HT. Compared with patients with NCA, patients with HGR had similar intima areas but smaller external elastic lamina areas (7.9 mm^2 [6.3, 11.2] versus 6.6 mm^2 [4.8, 7.5], p = 0.02) resulting in smaller lumen areas (4.5 mm^2 [3.4, 6.6] versus 3.3 mm^2 [2.8, 4.7], p = 0.04) in distal segments and smaller lumen diameters in side branches (1.28 mm [1.19, 1.37] versus 1.09 mm [0.94, 1.24], p = 0.04). Compared with patients with NCA, lesions in patients with HT were more homogeneous, involving the entire coronary vascular tree. Patients with HGR had a higher prevalence of macrophages involving ≥1 quadrant in all 3 segments compared with patients with NCA. The number of microvessels was greater in patients with both HGR and LGR HT versus NCA. In conclusion, distinct findings in the distribution and morphology of coronary lesions between HT recipients and patients with NCA are evident by OCT imaging, suggesting that OCT might be useful to help differentiate cardiac allograft vasculopathy from NCA in vivo. © 2016 Elsevier Inc. All rights reserved. (Am J Cardiol 2016; ■ ■ ■ ■)

Cardiac allograft vasculopathy (CAV), a major limitation to the long-term success of heart transplantation (HT), is still not completely understood in terms of pathologic mechanisms. Innate and adaptive immune responses are involved in the pathogenesis of CAV. Compared with traditional native coronary atherosclerosis (NCA), CAV has both histopathologic similarities and distinct differences. CAV is regarded as an accelerated diffuse fibroproliferative process that affects the entire coronary vascular tree; conversely, traditional NCA typically involves proximal coronary artery segments. Optical coherence tomography (OCT) provides high-resolution (10–20 μm) intravascular imaging in vivo to allow in vivo visualization of coronary artery microstructure, including macrophages and vasa vasorum in advanced atherosclerotic plaques, and measurements of intimal thickness. The main purpose of this study is to use OCT to compare the morphologic features of coronary lesions in transplanted hearts versus NCA.

Methods

From February 2011 to March 2015, 75 patients with HT at Columbia University Medical Center (New York, New York) were enrolled in the study. Fifteen subjects were excluded because of poor image quality secondary to incomplete blood washout during image acquisition (n = 7), previous stent implantation (n = 2), and non–left anterior descending (LAD) vessel imaging (n = 6). The remaining 60 subjects with OCT imaging of the LAD were included in the present study. Per protocol, all HT recipients underwent annual coronary angiography with right ventricular biopsy. Tissue rejection was graded according to the International Society of Heart and Lung Transplantation (ISHLT) classification proposed in 1990 and revised (R) in 2005^5; grade 0 (0R): no rejection; grade 1A (1R), focal, mild acute rejection; grade 1B (1R), diffuse, mild acute rejection; grade 2 (1R), focal, moderate acute rejection; grade 3A (2R), multifocal, moderate rejection; grade 3B (3R), diffuse, borderline severe acute rejection; and grade 4 (3R), severe acute rejection. Of the 60 HT recipients included in the present study, there were 17 subjects in the high-grade...
cellular rejection (HGR) group (ISHLT ≥3 A/2R) and 43 subjects in the none/mild-grade rejection (low-grade cellular rejection; LGR) group (ISHLT 0 to 2/0 to1R). The rejection grade used for this comparison was the worst rejection grade event recorded since HT.

In the same time period, we identified 60 patients with NCA without HT who underwent OCT examination of de novo LAD lesions for clinical purposes at the operator’s discretion at the time of diagnostic angiography (n = 22) or percutaneous intervention (n = 38).

The prespecified analysis was to compare patients with HGR with patients with NCA and patients with LGR with patients with NCA. The study was approved by the institutional review board, and all patients gave written informed consent.

Quantitative coronary angiography analysis was performed offline using QAngio XA, version 7.2.34.0 (Medis Medical Imaging Systems, Leiden, the Netherlands) without knowledge of OCT findings. After guiding catheter calibration, proximal, middle, and distal LAD segments were identified based on the American Heart Association classification that corresponded to the areas of OCT analysis. The minimal lumen diameter (MLD) and reference vessel diameter were measured; the diameter stenosis (DS) was calculated in each segment.

For acquisition of OCT images, the LightLab C7-XR Frequency Domain OCT system (St. Jude Medical, St. Paul, Minnesota) was used. After intracoronary nitroglycerin (100–200 μg), the OCT imaging catheter (Dragonyfly; St. Jude Medical) was advanced into the middle/distal segment of the LAD, and automatic pullback was initiated during continuous contrast injection (4 ml/s, 14–18 ml total), with a pullback speed of 10 to 25 mm/s. Offline analysis of OCT images was performed by 2 independent investigators (PS and AM) blinded to clinical characteristics using LightLab ORW software, version C.0.4 (LightLab; Westford, Massachusetts) at an independent core laboratory (Cardiovascular Research Foundation, New York, New York) according to previously described method.

After coregistration of OCT and angiographic studies, the OCT imaging runs were divided into 3 segments if all 3 (proximal, middle, and distal) angiographic segments had been visualized by OCT or 2 segments if only 2 (proximal and middle segments or middle and distal segments) angiographic segments had been visualized by OCT.

For quantitative analysis of each segment, we chose the frame with the minimum lumen area and the maximum plaque thickness (worst diseased site), and the frame with the maximum lumen area and the minimum plaque thickness (least diseased site), to measure external elastic lamina (EEL), internal elastic lamina (IEL), and lumen area for the distal segment but only the lumen area for the middle and proximal segments because of poor penetration in advanced plaques.

Percent media/EEL area, intima/EEL area, and intima/IEL area in the distal segments were calculated as follows:

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\text{Percent media/EEL area} = \frac{\text{EEL area } - \text{IEL area}}{\text{EEL area}} \times 100;
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\text{Percent intima/EEL area} = \frac{\text{IEL area } - \text{Lumen area}}{\text{EEL area}} \times 100;
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\text{Percent intima/IEL area} = \frac{\text{IEL area } - \text{Lumen area}}{\text{IEL area}} \times 100.
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For every visible side branch, the largest of the minimum diameters in consecutive OCT frames was measured.

The intima was considered eccentric if the minimal intimal thickness/maximal intimal thickness ratio was <0.5 or contained a lipid pool in ≥1 quadrants with normal intimal thickness (<0.3 mm) in the other quadrants. Microvessels within the intima appeared as signal-poor-poor tubular luminal structures without connection to the vessel lumen in ≥3 consecutive frames. The maximum number of microvessels (flow voids) was counted, and the presence of multiple (at least 3 microvessels) in any 1 frame was tabulated. Calcium was a signal-poor region with sharply delineated borders. Macrophage accumulation was characterized by increased signal intensity within the lesion accompanied by heterogeneous backward shadows and tabulated as involving ≥1 or <1 quadrant. Lipidic plaque was characterized by a signal-poor region with diffuse borders accompanied by an overlying signal-rich band.

Baseline patient clinical characteristics were analyzed on a patient level; and angiographic and OCT characteristics were analyzed on a segment (proximal, middle, and distal) level. For patient and segment level data, categorical variables are presented as frequencies and compared with chi-square statistics or Fisher’s exact test; continuous variables are presented as median and 1st and 3rd quartiles and compared using the Mann–Whitney U test. A model with the generalized estimating equations approach was used to compensate for any potential cluster effect of multiple sites in the same patient (e.g., multiple side branches, multiple calcium deposits, and microvessels) and presented as least square means with 95% CIs. Statistical analysis was performed with SAS software, version 9.1.3 (SAS Institute Inc., Cary, North Carolina). A probability value <0.05 was considered statistically significant. The prespecified end point was a comparison of NCA versus either HGR or LGR in patients with HT.

Results

OCT imaging of all 3 LAD segments was available in all patients with HGR and NCA, but OCT imaging of either the distal or proximal LAD segment was incomplete in 3 patients with LGR.

Compared with the combined group of patients with HGR and LGR HT, patients with NCA had a higher prevalence of hypertension, dyslipidemia, and a history of coronary artery disease, had a more favorable lipid profile and more aggressive antiplatelet therapy with clopidogrel, but a lower prevalence of renal insufficiency and lower left ventricular ejection fraction (Table 1). Half of the patients with NCA presented with an acute coronary syndrome (ACS); conversely, most transplant patients underwent routine invasive coronary imaging annually, and only 2 patients with HGR, but no patients with LGR presented with an ACS (p <0.01 for comparison between patients with NCA and the combined group of patients with HGR and LGR). Compared with patients with LGR, patients with HGR had longer intervals from transplantation to OCT imaging (p = 0.01) but similar donor age, cytomegalovirus reactivation and maintenance...
immunosuppression therapy, except for a trend for more sirolimus-based immunosuppression therapy ($p = 0.09$).

Angiographic findings are listed in Table 2. Compared with patients with NCA, patients with HGR had comparable values in the proximal LAD segment, larger MLDs and smaller DS in the middle segment, and smaller MLD and larger DS in the distal segment, whereas patients with LGR had larger MLDs in all 3 segments and smaller DS in both middle and proximal segments.

OCT quantitative findings are provided in Table 3, Figure 1, and Figure 2. At the maximally diseased site and compared with patients with NCA, patients with HGR had a similar lumen area in the proximal segment and a larger lumen area in the middle segment; however, patients with HGR had a smaller media area, comparable intima area, and smaller lumen area (because of a smaller EEL and IEL area) in the distal segment. Conversely, patients with LGR had a larger lumen area in all 3 segments as well as a smaller media area and intima area in the distal segment. At the minimally diseased sites, patients with HGR had no significant differences in the lumen area in all 3 segments, whereas patients with LGR had a larger lumen area in all 3 segments versus patients with NCA.

Qualitative OCT findings are shown in Figure 3. Compared with patients with NCA, patients with HGR had a lower prevalence of eccentric plaques, lipidic plaques, and calcium in both the proximal and middle LAD segments but no differences in the distal LAD segments. Conversely, patients with LGR had a lower prevalence of almost every qualitative OCT finding in all 3 segments compared with patients with NCA. Compared with patients with NCA, patients with HGR (but not patients with LGR) had a higher prevalence of macrophages involving $C_21$ quadrant in all 3 segments (Figure 3). Compared with 258 calcium deposits in patients with NCA, 29 calcium deposits in patients with HGR were more likely to be crescent-shaped, homogenous, and lack attenuation. These differences were not seen in 15 calcium deposits in patients with LGR (Figure 4). As a result, microvessels in patients with both HGR and LGR were more likely to be multiple, parallel to the lumen of the main vessel, and contained within the layered plaque versus patients with NCA in whom the pattern was more likely to be solitary, focal, and transverse through the plaque (Figure 4).
There were 542 analyzable side branches in patients with NCA, 133 side branches in patients with HGR, and 365 side branches in patients with LGR. Compared with patients with NCA, patients with HGR had smaller side branch lumen diameters (1.28 mm [1.19, 1.37] vs 1.09 mm [0.94, 1.24], p = 0.04), whereas patients with LGR had similar diameters (1.28 mm [1.19, 1.37] vs 1.26 mm [1.15, 1.36], p = 0.74; Figure 4).

Qualitative OCT findings—including eccentric plaques, macrophages involving ≥1 quadrant, microvessels, lipidic plaques and calcium—were more prevalent in the middle and proximal segments in patients with NCA. Conversely, there were no significant differences among the 3 segments in patients with either HGR or LGR (Figure 3).

Discussion

The principal findings of the present OCT study were: (1) compared with patients with NCA, lesions in patients with
HT were more homogeneous, involving the entire coronary vascular tree; (2) compared with patients with NCA, there was a smaller lumen area in the distal segment of HGR patients, probably due to negative remodeling; and (3) there was diffuse macrophage infiltration in all 3 (proximal, middle, and distal) LAD segments in patients with a history of HGR versus patients with NCA, a finding not seen in patients with LGR.

CAV is a multifactorial phenomenon with variable morphologic features including intimal fibromuscular hyperplasia, traditional atherosclerosis, and inflammation. The greatest disparity between CAV and NCA resides in initiating immunologic triggers in the allogeneic setting. In the present study, findings consistent with macrophage accumulation were more diffuse in patients with HGR, supporting the view that an inflammatory process may be a central event in CAV.

Figure 1. Quantitative distal (LAD) coronary artery analysis. OCT findings of EEL area and intimal area (left) and lumen area (right) in distal LAD coronary artery segments comparing patients with NCA, HGR, and LGR. The smaller lumen area in the HGR group compared to the NCA and LGR groups (right panel) is more likely due to a smaller EEL (left panel) than to a larger intimal area (middle panel).

Figure 2. Representative coronary angiography and OCT images of the LAD artery from patients with NCA, HGR, and LGR. Cross-sectional OCT images (right) from proximal to distal correspond to the white lines on the coronary angiograms (left). NCA (top): significant atherosclerotic characteristics are found in the proximal section (A) with TCFA (white asterisk) covered with white thrombus (white arrow) and in the middle section (B) with cholesterol crystal (white arrow) contained within lipidic plaque but not in the distal section (C) that shows a normal 3-layered vessel wall. HGR (middle): OCT images show diffuse intima thickening with an eccentric intima in the middle section (D) and concentric intima in both proximal (E) and distal (F) sections. LGR (bottom): OCT images show almost a normal, 3-layered vessel wall from proximal to distal sections (G to I). TCFA = thin-capped fibroatheroma.
CAV is a diffuse process characterized by concentric intimal thickening of both major epicardial and intramyocardial arteries with comparable severity from proximal to distal epicardial arteries and similar effects relative to gender and patient age. Conversely, NCA is usually focal, eccentric, involves more proximal large- and medium-sized coronary vessels, and increases with patient age. A dichotomous pattern of transplant coronary artery disease was previously suggested by grayscale intravascular ultrasound imaging and histological ex vivo studies: (1) diffuse, distal, and circumferential disease representing inflammatory-mediated vessel injury and (2) focal and noncircumferential proximal involvement similar to NCA and that may represent donor NCA transmission or development of new atherosclerosis. This distinction is consistent with our present study in which we saw more intimal thickening in the distal LAD and side branches of HGR HT recipients. In contrast, the development of atherosclerosis with vulnerable plaque and complicated coronary lesions (thin-capped fibroatheroma, well-formed lipid cores with cholesterol clefts, plaque ruptures, and intraluminal thrombus) were seen in patients with HT in a pattern similar to NCA. Furthermore, atherosclerotic characteristics were evenly distributed in the 3 segments in the transplant recipients compared with a more focal pattern in proximal and middle segments in NCA arteries. Finally, a smaller media area in patients with both HGR and LGR versus patients with NCA supported the view that CAV was associated with a preserved tunica media, whereas classical atherosclerotic lesions were characterized by disruption of IEL.

In our study of distal LAD segments, patients with HGR had the smallest lumen area, probably caused primarily by negative remodeling as evidenced by a smaller EEL and IEL area consistent with previous serial intravascular ultrasound studies showing that early lumen loss after HT was caused by intimal thickening and late lumen loss was caused by vessel constriction.14,15

Figure 3. Comparison of qualitative OCT findings. The frequency of each OCT finding—eccentric plaques, macrophages involving ≥1 quadrant, microvessels, lipidic plaque, and calcium—were compared between patients with NCA versus patients with HGR or LGR in the distal (top), middle (middle), and proximal (bottom) LAD coronary artery segments. A secondary comparison of proximal versus middle versus distal segments in each group (patients with NCA, HGR, and LGR) showed a higher prevalence of all these findings in the proximal and middle segments (vs distal segments) in patients with NCA (*p < 0.05) but not in patients with HGR or LGR.
Microvessels, tissue markers for plaque vulnerability, are widespread in the end stages of atherosclerotic coronary artery disease. The pattern of microvessels in HGR in the present study may suggest a pathogenic mechanism and clinical significance different from NCA: (1) a combination of a deep donor-transmitted atherosclerotic layer with superimposed superficial intimal proliferation due to CAV\textsuperscript{15} and (2) repeated episodes of mural thrombosis,\textsuperscript{16} as supported by a previous postmortem study demonstrating that coronary thrombosis is a frequent and diffuse finding in transplanted hearts with CAV.\textsuperscript{17} However, in the present study, there is no relation between the distribution of microvessels and macrophages.

Calcium is a major component of advanced atherosclerosis that can serve as a surrogate marker of disease severity. Our present study shows that calcium is significantly less common and had a different morphologic characterization in HT versus NCA. In NCA, calcium is most prevalent in fibroatheromas is mostly located around the necrotic core close to the media.\textsuperscript{18} Conversely, HT calcium is related to fibrotic intimal thickening, patients with HT have a greater ratio of calcified non—lipid-rich to calcified lipid-rich plaque compared to patients with NCA.\textsuperscript{9}

Although the present study included 60 patients with HT undergoing OCT examination, the study sample size was

**Figure 4. Calcium, microvessels, and side branches.** (A) A comparison of calcium, microvessels, and side branch diameters between NCA and patients with HGR or LGR. Values are displayed as n (%) or least square means with error bar (95% CI). \( \dagger p < 0.05 \); \( \ddagger p < 0.001 \). (B) Corresponding representative OCT images. Calcium (left): OCT shows block-shaped calcium with attenuation in NCA (a) but homogenous, new-moon—like shape in fibrotic plaque in HGR (b). Microvessels (middle): OCT shows a solitary microvessel on the shoulder of lipidic plaque in NCA (c) but multiple voids in fibrotic plaque parallel to the main lumen circumferentially in HGR (d). Side branches (right): OCT shows eccentric atherosclerotic plaque in the main vessel, sparing the side branch in NCA (e), but intimal hyperplasia involving a side branch in addition to the main vessel in HGR (f). White asterisks indicate calcium deposits; arrowheads represent microvessels; LP = lipidic plaque.
still relatively small. The NCA group included patients with both stable coronary artery disease and ACS presentation. This was a cross-sectional study of HT recipients undergoing routine coronary artery examination. OCT evaluation was performed only in the LAD. OCT evaluation was not performed at the time of HT; therefore, a clear differentiation between donor-transmitted NCA and de novo progressive CAV was not possible. OCT has technical limitations including poor tissue penetration that makes it difficult to measure plaque burden and identify lipid and calcium deposits behind a thick fibrous cap. Not all bright spots were necessarily caused by macrophages; some may have been caused by cholesterol crystals, elastin/collagen, and microcalcifications.19 Owing to a limited OCT pullback length, the very distal LAD was missed in some patients.

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