In vivo comparison between cardiac allograft vasculopathy and native atherosclerosis using near-infrared spectroscopy and intravascular ultrasound

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Aims
The aim was to compare cardiac allograft vasculopathy to native atherosclerosis by near-infrared spectroscopy-intravascular ultrasound (NIRS-IVUS).

Methods and results
Twenty-seven atherosclerotic (non-transplant) patients and 28 heart transplant recipients undergoing routine surveillance coronary angiography underwent NIRS-IVUS imaging of the left anterior descending coronary artery. In each proximal, middle, and distal coronary artery segment, the maxLCBI4mm [4-mm long segment with maximum lipid core burden index (LCBI)] and corresponding IVUS parameters were compared. MaxLCBI4mm was significantly greater among atherosclerotic patients than the transplant patients in both proximal and middle coronary artery segments, but not in the distal segment. There was a positive linear correlation between maxLCBI4mm and maximum plaque burden in both groups, but atherosclerotic patients demonstrated a smaller maxLCBI4mm than transplant recipients among segments with plaque burden ≥40%. Among segments with a maximum plaque burden ≥40%, native-atherosclerosis patients had a greater maxLCBI4mm compared with transplant patients (P = 0.015). Calcification was present in 72.9% of native atherosclerosis and 14.7% of transplant segments (P < 0.001). Among the 165 analysed segments, prevalence of lipid-rich plaque (LRP) with superficial attenuation (30.9 vs. 1.2%, P < 0.001) or calcified LRP (13.6 vs. 2.4%, P = 0.03) was significantly greater in native atherosclerosis compared with transplant patients. Conversely, the proportion of segments with non-LRP (46.4 vs. 11.1%, P < 0.001) was higher in transplant patients.

Conclusion
NIRS-IVUS imaging demonstrated early and accelerated lipid accumulation with smaller plaque burden and less calcium in patients after heart transplant when compared with native atherosclerosis.

Keywords
cardiac allograft vasculopathy • near-infrared spectroscopy • intravascular ultrasound • atherosclerosis

Introduction
With overall improvements in long-term survival of heart transplant patients, cardiac allograft vasculopathy (CAV) has become the leading cause of morbidity and mortality in these patients after the first year. Although the exact pathogenesis of CAV still remains unclear, CAV is considered to be an accelerated fibroproliferative process characterized by diffusely concentric vascular thickening of both the epicardial and intramuscular vasculature. In contrast, native atherosclerosis, which develops over several decades, is characterized as focal, non-circumferential, more eccentric, and more proximal than distal in location. Distinguishing between CAV and native atherosclerosis is still challenging. We used a combined intravascular ultrasound (IVUS) and near-infrared spectroscopy (NIRS) catheter
to assess both coronary artery structure and lipid-rich plaque (LRP) in vivo to compare CAV with native atherosclerosis.  

**Methods**

**Study population**

The analysis included all the patients who underwent coronary angiography and NIRS-IVUS imaging of the left anterior descending artery (LAD) between January 2013 and December 2013 at Columbia University Medical Center (New York, NY). Overall, we were able to analyse 28 heart transplant recipients who underwent routine surveillance coronary angiography and imaging and 27 patients with native atherosclerosis. Transplant patients underwent orthotopic cardiac transplantation between January 1994 and August 2012. The use of NIRS-IVUS during routine surveillance coronary angiography for transplant patients was protocol defined; for the native-atherosclerosis group, it was at the operator’s discretion. The study was approved by the institutional review board, and all patients gave written informed consent.

**Quantitative coronary angiography analysis**

Off-line angiographic analysis was done using QAngio XA version 7.2.34.0 (Medis Medical Imaging Systems, Leiden, the Netherlands) blinded to the clinical and NIRS-IVUS findings. Using the contrast-filled guiding catheter for calibration, the proximal, middle, and distal coronary artery segments were identified that corresponded to the NIRS-IVUS analysis. The minimum lumen diameter (MLD) and reference vessel diameter (RVD) in each segment were measured, and the diameter stenosis (DS) was calculated. The minimum lumen diameter (MLD) and reference vessel diameter (RVD) in each segment were measured, and the diameter stenosis (DS) was calculated.6

**NIRS-IVUS image acquisition and analysis**

After intracoronary administration of nitroglycerine (100–200 µg), the NIRS-IVUS imaging catheter (TVCA Imaging System, InfraRedx, Burlington, MA) was advanced as distally as possible in the target vessel; motorized pullback was performed at 0.5 mm/s until reaching the aorta.

IVUS images were analysed off-line by two independent investigators (B.Z. and A.M.) using computerized planimetry software (echoPlaque 4.0, INDEC Medical Systems, Inc., Santa Clara, CA). After co-registration of IVUS and angiographic studies and assessment of the angiograms based on the American Heart Association classification of proximal, middle, and distal segments, the IVUS imaging run was divided into three equal segments if all three (proximal, middle, and distal) angiographic segments had been visualized by IVUS or two equal segments if only two angiographic segments (proximal and middle segments or middle and distal segments) had been visualized by IVUS.7,8

The NIRS-IVUS imaging catheter enables simultaneous NIRS and IVUS image acquisition. The NIRS chemogram displays the distribution of the probability of LRP with the X-axis indicating the pullback position (1 pixel every 0.1 mm) and the Y-axis indicating the circumferential position (1 pixel every 1°). Lipid core burden index (LCBI), the fraction of pixels indicating lipid within a region of interest, was calculated as pixels with a probability of LRP > 0.6 divided by all viable pixels multiplied by 1000.9–12 Total vessel LCBI and maximum LCBI in any 4-mm region (maxLCBI<sub>4mm</sub>) for each proximal, middle, and distal segment were analysed off-line using Matlab-based software.13

Cross-sectional IVUS measurements included external elastic membrane (EEM) area, lumen area, maximum and minimum intimal thicknesses, and plaque burden (EEM area—lumen area)/EEM area • 100, %). The maximal plaque burden within each segment was determined as follows: if there was no LRP (maxLCBI<sub>4mm</sub> = 0), the maximal plaque burden within the segment was determined; in the presence of LRP (maxLCBI<sub>4mm</sub> > 0), the maximal plaque burden within the 4-mm long segment with the largest amount of LRP was determined. Calcification by IVUS was defined by the presence of a bright echogenic signal with acoustic shadowing.14,15 Attenuated plaque was identified by the absence of the ultrasound signal behind plaque that was either hypoechoic or isoechoic to the reference adventitia but contained no bright calcification.13 Superficial attenuation was defined as the leading edge of attenuation closer to the lumen than to the adventitia.16

According to the presence of LRP, superficial attenuation, and calcium, we categorized plaques as (1) non-LRP, (2) LRP without superficial attenuation, (3) LRP with superficial attenuation, (4) calcified LRP, and (5) calcified non-LRP.

**Statistical methods**

Statistical analysis was performed with SAS version 9.1 (SAS Institute, Cary, NC). For patient-level data, categorical variables are presented as frequencies and compared with χ² statistics or Fisher’s exact test (if there was an expected cell value < 5); continuous variables are presented as median and interquartile range and compared using Mann–Whitney U test. While pooling the data of all three segments together, a model with a generalized estimating equation (GEE) approach was used to compensate for any potential cluster effect of multiple segments in the same individual. The correlation between IVUS-derived parameters and NIRS-derived parameters was analysed using Spearman’s regression coefficients, and the interaction between different group and IVUS parameters was evaluated. To identify independent predictors of maxLCBI<sub>4mm</sub> clinical variables with P < 0.2 were entered into the clinical multiple linear regression model. A P < 0.05 was considered statistically significant.

**Results**

**Clinical characteristics**

Patient characteristics are summarized in Table 1. Native-atherosclerosis patients were older and had a history of coronary artery disease with lower lipid levels than the transplant patients. There were no other significant clinical differences between the groups. For the transplant recipients, the time from transplant was 7.0 ± 5.5 (median 4.5) years. The incidence of immunosuppressive agents use, including steroids, tacrolimus, sirolimus, mycophenolate mofetil, mycophenolic acid, and cyclosporine was 82.1, 64.3, 7.1, 67.9, 7.1, and 28.6%, respectively. According to the International Society of Heart and Lung Transplantation (ISHLT) classification in 1990 and 2005,17 the acute cellular rejection was graded as follows: Grade 0: 10.7%, Grade 1A-1R: 57.1%, Grade 1B-1R: 0%, Grade 2-IR: 3.6%; Grade 3A-2R: 25.0%, and Grade 3B-2R: 3.6%. In the atherosclerosis group, 1 patient (3.7%) presented with non-ST-segment elevation myocardial infarction, 18 patients (66.7%) with unstable angina, 7 (25.9%) with stable angina, and additional 2 with silent myocardial ischaemia. The majority of transplant patients underwent routine invasive coronary imaging annually, and only one patient presented with angina (P < 0.001). In addition, most of the transplant recipients (96.4%) did not receive target vessel (LAD) intervention; however, more than two-thirds of patients in the atherosclerosis group underwent LAD intervention (P < 0.001).
Angiographic findings

Angiographic findings are shown in Table 2. The MLD and the RVD were significantly smaller, and the DS was significantly higher in the native-atherosclerosis group vs. the transplant group.

Quantitative IVUS findings

Representative cases from each of the two groups are shown in Figure 1. In total, 13.6% (11/81) of segments with a maximum plaque burden <40% and 86.4% (70/81) of segments with a plaque burden ≥40% were identified in the native-atherosclerosis group; conversely, there were 71.4% (60/84) of segments with a maximum plaque burden <40% and 28.6% (24/84) of segments with a plaque burden ≥40% in the transplant group (P = 0.001).

Quantitative greyscale IVUS findings are shown in Figure 2. There were no significant differences in proximal, middle, and distal EEM areas between the native-atherosclerosis and transplant groups; however, plaque areas and maximum intimal thicknesses were significantly higher in the native-atherosclerosis group, resulting in smaller lumen areas in all three segments.

Comparing the three segments (proximal, middle, and distal) in the two groups separately, plaque burden was not significantly different among the three segments (P = 0.07) in the transplant group; however, in the native-atherosclerosis group, there was a gradient from proximal and middle segments to the distal (P = 0.03).

NIRS findings

For the entire vessel, LCBI was significantly greater in native-atherosclerosis patients compared with transplant recipients [88 (43, 122) vs. 16 (1, 59), P < 0.001]. Similarly, the maxLCBI<sub>4mm</sub> was greater in the native-atherosclerosis vs. the transplant patients in the proximal and middle segments, but not in the distal segment [proximal: 209 (112, 328) vs. 26 (0, 145), P < 0.001; middle: 215 (45, 390) vs. 6 (0, 175), P < 0.001; and distal: 38 (0, 305) vs. 0 (0, 149), P = 0.16, respectively (Figure 2)].

Comparing proximal vs. middle vs. distal segments in the two groups separately, there was no significant difference in maxLCBI<sub>4mm</sub> among the three segments in either the native-atherosclerosis (P = 0.36) or transplant group (P = 0.85).

### Table 1  Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Transplant vasculopathy (n = 28)</th>
<th>Native atherosclerosis (n = 27)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>60 (44–65)</td>
<td>63 (56–76)</td>
<td>0.02</td>
</tr>
<tr>
<td>Male</td>
<td>71.4% (20)</td>
<td>85.2% (23)</td>
<td>0.33</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.6 (25.6–33.8)</td>
<td>26.7 (24.4–31.5)</td>
<td>0.41</td>
</tr>
<tr>
<td>Prior CAD</td>
<td>50% (14)</td>
<td>81.5% (22)</td>
<td>0.02</td>
</tr>
<tr>
<td>Prior MI</td>
<td>14.3% (4)</td>
<td>18.5% (5)</td>
<td>0.73</td>
</tr>
<tr>
<td>PVD</td>
<td>3.6% (1)</td>
<td>7.4% (2)</td>
<td>0.61</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>46.4% (13)</td>
<td>25.9% (7)</td>
<td>0.16</td>
</tr>
<tr>
<td>Hypertension</td>
<td>67.9% (19)</td>
<td>77.8% (21)</td>
<td>0.55</td>
</tr>
<tr>
<td>Smoking</td>
<td>32.1% (9)</td>
<td>59.3% (16)</td>
<td>0.06</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>78.6% (22)</td>
<td>81.5% (22)</td>
<td>1.0</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>173.0 (145.5–208.5)</td>
<td>141.0 (120.3–177.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>129.0 (96.0–174.5)</td>
<td>118.5 (84.8–143.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>46.0 (34.5–57.5)</td>
<td>38.0 (30.8–48.5)</td>
<td>0.045</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>97.5 (73.5–111.75)</td>
<td>79.5 (64.0–100.5)</td>
<td>0.07</td>
</tr>
<tr>
<td>LDL-C/HDL-C ratio</td>
<td>2.1 (1.5–2.6)</td>
<td>2.3 (1.6–2.7)</td>
<td>0.60</td>
</tr>
<tr>
<td>Statin use</td>
<td>75% (21)</td>
<td>88.9% (24)</td>
<td>0.30</td>
</tr>
<tr>
<td>Aspirin use</td>
<td>75% (21)</td>
<td>88.9% (24)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Values are percentage (number) or median (interquartile range). CAD, coronary artery disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; PVD, peripheral vessel disease.

### Table 2  Quantitative angiographic findings

<table>
<thead>
<tr>
<th></th>
<th>Transplant vasculopathy (n = 28)</th>
<th>Native atherosclerosis (n = 27)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal segment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVD, mm</td>
<td>3.5 (3.2–3.8)</td>
<td>3.3 (2.9–3.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>MLD</td>
<td>3.5 (3.2–3.8)</td>
<td>2.7 (2.2–3.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DS, %</td>
<td>5.6 (2.9–7.5)</td>
<td>6.4 (4.5–18.2)</td>
<td>0.06</td>
</tr>
<tr>
<td>Middle segment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVD, mm</td>
<td>3.0 (2.6–3.3)</td>
<td>2.6 (2.4–3.0)</td>
<td>0.14</td>
</tr>
<tr>
<td>MLD</td>
<td>2.6 (2.2–2.9)</td>
<td>2.2 (1.7–2.6)</td>
<td>0.007</td>
</tr>
<tr>
<td>DS, %</td>
<td>8.6 (5.3–13.6)</td>
<td>15.1 (10.9–24.1)</td>
<td>0.007</td>
</tr>
<tr>
<td>Distal segment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVD, mm</td>
<td>2.5 (2.3–2.8)</td>
<td>2.2 (2.0–2.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>MLD</td>
<td>2.2 (2.0–2.7)</td>
<td>1.8 (1.6–2.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>DS, %</td>
<td>6.0 (3.5–11.4)</td>
<td>11.5 (6.1–18.8)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Values are median (interquartile range). DS, diameter stenosis; MLD, minimum lumen diameter; RVD, reference vessel diameter.
By regression analysis, there was a moderate correlation between maximum plaque burden vs. maxLCBI_{4mm} by pooling all segments together in the native-atherosclerosis patients ($R = 0.51, P < 0.001$) and a weak correlation in the transplant recipients ($R = 0.34, P = 0.03$) (Figure 3A and B). However, the two regression lines intersect at a value of 40% plaque burden (interaction $P = 0.015$) such that among segments with plaque burden $< 40\%$, the maxLCBI_{4mm} was greater in transplant patients than in native-atherosclerosis patients while among segments with a maximum plaque burden $\geq 40\%$, native-atherosclerosis patients had a greater maxLCBI_{4mm} compared with transplant patients (Figure 3C).
Calcification was present more often in the native-atherosclerosis than in the transplant group (72.9 vs. 14.7%, \( P_{0.001} \)). Among the overall group of 165 analysed segments, native-atherosclerosis patients had higher prevalence of LRP with superficial attenuation (30.9 vs. 1.2%, \( P_{0.001} \)) and calcified LRP (13.6 vs. 2.4% \( P_{0.03} \)) when compared with transplant vasculopathy patients. Conversely, the proportion of segments with non-LRP was smaller in the native-atherosclerosis than in the transplant group (11.1 vs. 46.4% \( P_{0.001} \)) (Figure 4).

In contrast, CAV has been considered a process involving immunologic and non-immunologic mechanisms, different from common atherosclerotic coronary artery disease.\(^2\) Several studies have revealed evidence of lipid accumulation, including clustering of lipid-rich inflammatory cells and extracellular lipid deposition, even in the early stages of CAV.\(^2\)\(^ \^{4,20} \) MacManus et al. have demonstrated that lipid accumulation is important, early, and persistent in the development of transplant vasculopathy with lipids, even in lesions with <25% narrowing, that was not associated with duration since heart transplant.\(^2\)\(^4\)

In the current study, plaque burden was positively associated with the amount of lipid assessed by NIRS-IVUS in both transplant and native-atherosclerosis patients; however, the current study showed that within segments with a plaque burden <40%, maxLCBI\(_{\text{mm}} \) was significantly greater in the transplant group than in the native-atherosclerosis group, indicating very early and accelerated lipid accumulation in transplanted coronary arteries. Further investigation is needed to determine whether and how early lipid accumulation with mild plaque hyperplasia will impact adverse events in transplant patients.

**Coronary artery plaque morphology evaluated by NIRS-IVUS**

Greyscale IVUS attenuated plaque has been associated with the clinical presentation of ST-segment-elevated myocardial infarction and adverse clinical events, including periprocedural myocardial infarction and no-reflow, during percutaneous coronary intervention.\(^2\)\(^1\)\(^–\)\(^2\)\(^3\) Recently, Pu et al.\(^1\)\(^6\) reported that attenuated plaque, especially superficial attenuated plaque, was associated with LRP with necrotic core. However, there is no literature regarding attenuated plaque in heart transplant recipients. In the current study, the prevalence of NIRS-LRP with superficial greyscale IVUS plaque attenuation was
significantly greater among atherosclerotic patients compared with transplant recipients, whereas the incidence of LRP without superficial attenuated plaque was comparable between the two groups, reflecting a different stage of plaque development as well as a different circumferential lipid distribution, which might be more diffuse and deeper among transplanted coronary vessels compared with native atherosclerotic arteries. Kolodgie et al.24 pointed out that lipid accumulation was more likely to be present in the deep intimal layer with diffuse distribution between smooth muscle cells, representing the early stage of lesion growth—i.e. pathologic intimal thickening—that is unlikely to cause superficial attenuated plaque by greyscale IVUS.16 On the other hand, previous pathologic studies revealed early, diffuse, intimal, and medial lipid deposits in cardiac allograft coronary arteries.2,4

As a signature of native atherosclerosis, calcification is found more frequently in advanced stages. Furthermore, the deposition of both calcium and lipids may be present in most advanced atherosclerotic lesions.25 In terms of transplant coronary disease, the deposition of calcium was rarely observed in the early stages, and the prevalence and amount of calcium deposit increased over time after transplantation.26–28 In the present study, the incidence of calcification and the proportion of calcified LRP was significantly higher among native-atherosclerotic patients, consistent with more advanced plaque progression compared with transplant recipients.

Most studies of lesion composition in CAV have, out of necessity, been conducted in histologic studies post-mortem or after explants. While these studies permit an accurate histologic assessment of lesions, they provide data at a single time point and cannot assess results of therapy in which baseline and follow-up measures are needed. In contrast, the NIRS-IVUS instrument can be used to obtain serial measurements of lipid content and plaque burden in living patients. Thus, NIRS-IVUS imaging could be a useful approach for evaluation of disease progression and response to existing and novel therapies.

**Limitations**

The sample size was relatively small. Therefore, type I statistical error may have occurred. Due to the absence of NIRS-IVUS evaluation at the time of the transplant, we could not eliminate the possibility of donor atherosclerosis. The current study included both culprit and non-culprit vessels, and only the LAD artery was studied. We selected plaque burden ≥40% as a cut-off to discriminate advanced disease from early-stage lesion.29 However, the number of the lesions with a plaque burden of <40% in the atherosclerosis group was relatively low, whereas a limited number of lesions with a plaque burden of ≥40% were observed in the transplant group. A previous report demonstrated that a maxLCBI_{trans} could be a signature of culprit lesions among STEMI patients.5 In addition, Oemrawsingh et al.30 reported that CAD patients with an LCBI equal to or above the median of 43.0, as assessed by NIRS in a non-culprit coronary artery, had a four-fold risk of adverse cardiovascular events during 1-year follow-up. However, as a novel device, the validation of a
relatively low LCBI by NIRS as well as the feasibility for assessment of very distal coronary arteries—especially among transplant recipients with CAV—has not been fully investigated. Further investigation is needed to compare the amount of lipid among lesions with similar plaque burden between transplant and traditional atherosclerotic patients in a larger population. Also, the early-stage lipid deposition of CAV detected by NIRS-IVUS should be validated by histopathological analysis.

Conclusions

NIRS-IVUS imaging demonstrated early and accelerated lipid accumulation with a smaller plaque burden and less calcium among patients after heart transplantation compared with patients with native atherosclerosis.

Acknowledgement

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Conflict of interest


References