Volumetric Characterization of Human Coronary Calcification by Frequency-Domain Optical Coherence Tomography

Emile Mehanna, MD; Hiram G. Bezerra, MD, PhD; David Prabhu, BSc; Eric Brandt, MD; Daniel Chamié, MD; Hirosada Yamamoto, MD; Guilherme F. Attizzani, MD; Satoko Tahara, MD, PhD; Nienke Van Ditzhuijzen, BSc; Yusuke Fujino, MD; Tomoaki Kanaya, MD; Gregory Stefano, MD; Wei Wang, PhD; Madhusudhana Gargesha, PhD; David Wilson, PhD; Marco A. Costa, MD, PhD

**Background:** Coronary artery calcification (CAC) presents unique challenges for percutaneous coronary intervention. Calcium appears as a signal-poor region with well-defined borders by frequency-domain optical coherence tomography (FD-OCT). The objective of this study was to demonstrate the accuracy of intravascular FD-OCT to determine the distribution of CAC.

**Methods and Results:** Cadaveric coronary arteries were imaged using FD-OCT at 100-μm frame interval. Arteries were subsequently frozen, sectioned and imaged at 20-μm intervals using the Case Cryo-Imaging automated system™. Full volumetric co-registration between FD-OCT and cryo-imaging was performed. Calcium area, calcium-lumen distance (depth) and calcium angle were traced on every cross-section; volumetric quantification was performed offline. In total, 30 left anterior descending arteries were imaged: 13 vessels had a total of 55 plaques with calcification by cryo-imaging; FD-OCT identified 47 (85%) of these plaques. A total of 1,285 cryo-images were analyzed and compared with corresponding co-registered 257 FD-OCT images. Calcium distribution, represented by the mean depth and the mean calcium angle, was similar, with excellent correlation between FD-OCT and cryo-imaging respectively (mean depth: 0.25±0.09 vs. 0.26±0.12 mm, P=0.742; R=0.90), (mean angle: 35.33±21.86° vs. 39.68±26.61°, P=0.207; R=0.90). Calcium volume was underestimated in large calcifications (3.11±2.14 vs. 4.58±3.39 mm³, P=0.001) in OCT vs. cryo respectively.

**Conclusions:** Intravascular FD-OCT can accurately characterize CAC distribution. OCT can quantify absolute calcium volume, but may underestimate calcium burden in large plaques with poorly defined abluminal borders. (Circ J 2013; 77: 2334–2340)

**Key Words:** Coronary artery calcification; Cryo-imaging; Optical coherence tomography; Percutaneous coronary intervention
Volumetric Characterization of Coronary Calcium by FD-OCT

FD-OCT Image Acquisition

FD-OCT imaging was performed within 48 h of the donor’s death. Each vessel was mounted on a specially designed imaging rig and a plastic luer-lock was inserted and fixed at the ostium of the coronary artery. Residual luminal blood was flushed using normal saline solution (0.9% NaCl). All major side branches were sutured closed using 3-O silk. Vessels were then pressurized at room temperature using optimal cutting temperature compound (refractive index=1.439) (Tissue Tek, Ted Pella, Inc, Redding, CA, USA). A standard floppy 0.014-inch angioplasty guidewire was inserted through the ostium and positioned in the distal part of the vessel. The FD-OCT catheter (Dragonfly™, St Jude Medical) was advanced over the guidewire to the most distal part of the vessel. The FD-OCT Imaging System (C7-XR, St Jude Medical) was used, and multiple images were acquired in order to include the entire artery. A pullback speed of 10.0 mm/s was used, yielding a frame interval of 100 μm.

Cryo-Imaging

Immediately after OCT imaging, the entire rig was filled with optimal cutting temperature gel, covered with aluminum foil, and snap frozen by placing it in a container filled with liquid nitrogen. The frozen specimen block was then stored at –80°C in preparation for cryo-imaging. Frozen specimens were cut into 3–5 cm blocks, and placed in the cryo-imaging system to allow equilibration to the –20°C cutting temperature. The Case cryo-imaging system has been previously described, Briefly, it consists of a modified, large-section cryo-microtome (8250 Large Section Cryostat, Vibratome, St. Louis, MO, USA), XYZ robotic positioner carrying an imaging system that com-

Methods

Study Design and Sample Collection

Coronary vessels were obtained from the Cuyahoga County Examiner’s office from human cadavers within 24 h of death. Vessels were selected according to age (males >55 years; females >65 years), sex and the presence of risk factors for atherosclerosis (known history of hypertension, diabetes mellitus or hypercholesterolemia). Specimens were stored at 4°C prior to FD-OCT imaging. This procedure was deemed in accordance with federal, state, and local laws by the Case Institutional Review Board.

Figure 1. Coronary artery calcification (CAC) by frequency-domain optical coherence tomography (FD-OCT) and cryo-imaging. Consecutive bright field (1st row) and fluorescent (2nd row) cryo-images visualized with the corresponding FD-OCT images (3rd row) prior to quantification. Note: CAC shows poor visualization of abluminal calcium borders by FD-OCT.
FD-OCT Analysis

FD-OCT images were analyzed at the University Hospitals Cardiovascular Imaging Core Laboratory, by an independent analyst blinded to the cryo-images, using commercially available offline analysis software (Version C.0.4, St Jude Medical). All tracings were reviewed by a second, blinded analyst and a third analyst was consulted in cases of disagreement. Calcium area was determined by manual segmentation following the sharply defined edges of the signal-poor calcified region. Whenever the abluminal calcium border could not be identified, “anchor” points were positioned at the deepest identifiable side edges, and automatic software interpolation was used for determination of the calcium abluminal border. Based on this methodology, calcified plaques seen by FD-OCT were separated into 2 groups according to the ability to identify the abluminal calcium border (group A) or requirement to interpolate tracings in cases where the abluminal border was not visualized because of light attenuation in large calcifications (group B). Calcium angle was traced from the center of the lumen (the geometric center (centroid) of the traced lumen) using the functionality available in the commercially available software that we used. Depth of calcium was defined as the distance between the luminal border of the calcific plaque to the lumen contour and was determined at 1 degree circumferential intervals (Figure 2). Calcium volume was calculated using Simpson’s rule and measurements from every single frame of the entire plaque.

Calcified Plaque Identification

FD-OCT and cryo-images were visualized and reviewed using Amira software (Mercury Computer Systems Inc, Chelmsford, MA, USA). Proper co-registration between the 2 imaging modalities was performed using fiduciary marks, side branch location and plaque shape. Calcified plaques were identified using previously well-established plaque identification criteria (Figure 1).

Cryo-Image Analysis

Vessel lumen, and calcium area, angle and depth were manually segmented. The segmented datasets were exported and analyzed by Matlab-based software developed in our institution that automatically detects lumen center and measures plaque angle based on the widest pixel marked as containing calcified plaque. Plaque area was then determined for each slice, and plaque volume was calculated using Simpson’s rule.
Volumetric Characterization of Coronary Calcium by FD-OCT

Mixed-effects model was used to estimate the correlation coefficient between measurements from 2 methodologies. Bland-Altman plots were used to further evaluate 2 measurements.

**Figure 3.** Lipid masking human coronary artery calcification on FD-OCT. Bright-field and fluorescent cryo-images with corresponding OCT images showing 2 spotty calcifications (arrows in A, D and G) in the proximal coronary segment. Light attenuation caused by lipid is observed in FD-OCT images (arrowheads in H), which prevents visualization of corresponding calcification (arrowheads in B and E). Distal calcium in the same vessel is clearly visualized in both cryo and FD-OCT (arrows in C, F and I) images. FD-OCT, frequency-domain optical coherence tomography.

**Table.** Comparison of Calcium-Lumen Distance, Mean and Maximum Calcium Angles and Calcium Volume From FD-OCT and Cryo-Imaging of Calcified Plaques

<table>
<thead>
<tr>
<th></th>
<th>OCT</th>
<th>Cryo</th>
<th>Diff (OCT-Cryo)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean calcium-lumen distance, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n=19)</td>
<td>0.25±0.09</td>
<td>0.26±0.12</td>
<td>–0.00±0.06</td>
<td>0.742</td>
</tr>
<tr>
<td>Mean calcium angle, degrees</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n=19)</td>
<td>35.33±21.86</td>
<td>39.68±26.61</td>
<td>–4.35±11.88</td>
<td>0.207</td>
</tr>
<tr>
<td>Calcium volume, mm³</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n=19)</td>
<td>1.42±1.86</td>
<td>1.99±2.87</td>
<td>–0.57±1.70</td>
<td>0.150</td>
</tr>
<tr>
<td>Group A (n=12)</td>
<td>0.43±0.53</td>
<td>0.47±0.64</td>
<td>–0.04±0.15</td>
<td>0.450</td>
</tr>
<tr>
<td>Group B (n=7)</td>
<td>3.11±2.14</td>
<td>4.58±3.39</td>
<td>–1.47±2.68</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Group A, plaques with all calcium borders visualized on FD-OCT; Group B, plaques with poor visualization of abluminal calcium borders by FD-OCT, requiring interpolation in tracings. FD-OCT, frequency-domain optical coherence tomography.

**Statistical Analysis**

Analysis was conducted using SAS 9.2 (SAS Institute Inc, Cary, NC, USA). Continuous variables are presented as mean±SD. The difference between FD-OCT and cryo-imaging measurements was evaluated by linear mixed-effects model with random intercept (to account for within-subject correlation) and with methodology (Cryo and FD-OCT) as fixed effects. Mixed-effects model was used to estimate the correlation coefficient between measurements from 2 methodologies. Bland-Altman plots were used to further evaluate 2 measurements.
from Cryo and FD-OCT modalities.

Results

FD-OCT and cryo-imaging was performed in 30 left anterior descending arteries. Of these, 13 vessels had a total of 55 calcified plaques in 28 different segments identified by cryo-imaging. A total of 24,390 FD-OCT cross-sectional images were screened for the presence of CAC. FD-OCT identified 47 calcified plaques (85.4% sensitivity) in 13 vessels with co-registered vessel segments. All 47 calcified plaques seen by FD-OCT had CAC by cryo-imaging (100% specificity). A total of 8 calcified plaques were not identified by FD-OCT: 6 of 8 plaques had superficial lipid, which attenuated the light signal and prevented visualization of deeper calcium (Figure 3); 2 of 8 plaques had very small CAC that was likely missed because of the 100-μm sampling interval; 3 calcified plaques identified by FD-OCT were excluded because they were partially obscured by the guidewire shadow, precluding quantification. In addition, because the used cryo-imaging machine limits the

![Figure 4](image-url)

**Figure 4.** Correlations graphs (A) and Bland-Altman plots (B) between FD-OCT and cryo-imaging measurements of calcium-lumen distance, mean calcium angle and calcium volume. Correlation graphs and Bland-Altman plots for calcium-lumen distance (a), mean calcium angle (b), calcium volume for: the entire population (c), group A with fully visualized calcium borders (d) and group B with poor visualization of abluminal calcium border (e). FD-OCT, frequency-domain optical coherence tomography.
Volumetric Characterization of Coronary Calcium by FD-OCT

expand on previous observations and confirm the cross-sectional and volumetric appearance of CAC as sharply-delineated, signal-poor images on FD-OCT. Our study also introduces a new methodology, namely cryo-imaging, to enable co-registration between imaging modalities and assessment of entire volumes of coronary segments (Figure 5). Using a commercially available platform to analyze FD-OCT images, we were able to perform a comprehensive geometric characterization of CAC that included distribution (depth and angulation) and volumetric quantification of calcium burden. Overall, there was good agreement between FD-OCT and cryo-imaging, particularly when all borders of the calcified plaque could be delineated.

The ability of FD-OCT to characterize atherosclerotic plaques lies beyond a binary determination of the presence or absence of CAC. Other imaging modalities have been limited by poor spatial resolution, and high reflection (blooming) and shadowing generated by CAC. High spatial resolution coupled with the ability of near-infrared light to penetrate calcium enabled accurate determination of the distance between the lumen surface and intramural calcification (ie, the depth of calcium), irrespective of its extent. Further, high-speed imaging minimizes motion artifact and enables 3D image reconstruction.

The presence of a calcified plaque in direct contact with the arterial lumen poses significant challenges to interventional cardiologists. Debunk technologies, such as rotational atherectomy, have been specifically developed to ablate calcium and facilitate delivery of coronary devices. In this context, the characterization of the depth of calcium within the vessel wall may have profound clinical implications. Our group and others have described the use of FD-OCT for assessment of calcium modification by debunking techniques. The present report provides fundamental scientific validation supporting a potential future role of FD-OCT in characterizing calcium during coronary interventions in humans. On the other hand, our study also showed that the presence of superficial lipid within

Calcium Distribution
The mean depth of CAC was 0.25±0.09 mm vs. 0.26±0.12 mm in OCT and cryo, respectively (P=0.742), and the mean CAC angle was 35.3±21.8° vs. 39.6±26.6° (P=0.207) respectively (Table). There was also good correlation and agreement between the 2 modalities (Figure 4).

Calcium Volume Determination
The abluminal calcium border was clearly identified in 12/19 plaques (63%), whereas interpolation was required to trace the entire CAC outer border in 7/19 plaques (37%). Calcium volumes were similar as measured by FD-OCT and cryo-imaging in the group with visible abluminal border (0.43±0.53 mm³ vs. 0.47±0.64 mm³, respectively, P=0.450). There was excellent correlation for volume determination between the 2 imaging modalities in this subgroup (R=0.989, P<0.001). FD-OCT yielded smaller calcium volumes than cryo-imaging when the distal border of the calcium was not visualized and interpolation in the tracings was required (3.1±2.14 mm³ vs. 4.58±3.39 mm³, respectively, P<0.001). There was poor correlation for calcium volume calculations between FD-OCT and cryo-imaging (R=0.593, P=0.180) when interpolation was required.

Discussion
The present report provides the first validation of FD-OCT for characterizing the distribution of CAC in humans.
a plaque, and the subsequent light attenuation, prevented both qualitative and quantitative assessment of plaque components deeper into the vessel wall in 6 plaques. The clinical implication of potential underestimation of CAC in such cases is not well understood.

There was overall good agreement between FD-OCT and cryo-imaging quantification of CAC volume and circumferential distribution. However, the deep, large calcified plaques required interpolation of contours based on the edges of the deepest visualized calcification. As a result, underestimation of CAC volume occurred for such plaques, which represented 37% of our study sample. Kume et al previously observed good correlation between prior generation TD-OCT and standard histology, although calcium area was underestimated by TD-OCT. Quantification of CAC distribution, as well as volume, in plaques with well-defined borders, representing 63% of the plaques analyzed in the present study, showed very high accuracy. Although our results are promising, future clinical studies are warranted to determine if better visualization and quantification of CAC by FD-OCT will ultimately improve procedural and long-term outcomes in clinical practice.

Study Limitations

The major limitation of the present study is the relatively small sample size. However, the high number of images/frames analyzed for every plaque enabled collection of multiple data points for the parameters of interest. Another limitation is the size of the imaged blocks by cryo (3–5 cm). Nevertheless, this size limit does not affect the accuracy of quantification of the 19 plaques included in our study and is being currently addressed in the second-generation of the cryo-machine, which aims at imaging larger blocks.

Conclusion

Intravascular FD-OCT can accurately characterize CAC distribution; conversely, absolute calcium volume in large plaques with poorly defined abluminal borders may be underestimated.

Disclosures

Marco A. Costa and Hiram G. Bezerra receive honoraria and research grants from St Jude Medical Inc. Guilherme F. Attizzani receives consulting fees from St Jude Medical Inc., David Wilson has a financial interest in BioVision Inc, which intends to commercialize cryo-imaging. Emile Mehanna, Eric Brandt, David Prabhu, Daniel Chami, Hirosada Yamamoto, Satoko Tahara, Nienke Van Ditzhuijzen, Yusuke Fujino, Tomoaki Kanaya, Gregory Stefano, Wei Wang, and Madhushudana Gargesha do not have any conflicts of interest to declare.

References