Atherosclerotic Plaque Composition and Classification Identified by Coronary Computed Tomography

Assessment of Computed Tomography–Generated Plaque Maps Compared With Virtual Histology Intravascular Ultrasound and Histology

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Background—Computed tomography (CT) is used routinely for coronary angiography, and higher-risk features of plaques can also be identified. However, the ability of CT to discriminate individual plaque components and classify plaques according to accepted histological definitions is unknown.

Methods and Results—We first determined CT attenuation ranges for individual plaque components using combined in vivo and ex vivo CT coregistered with virtual histology intravascular ultrasound (VH-IVUS) in 108 plaques from 57 patients. Comparison with contrast attenuation created plaque/contrast attenuation ratios that were significantly different for each component. In a separate validation cohort of 47 patients, these Plaque Maps correlated significantly with VH-IVUS–determined plaque component volumes (necrotic core: \( r=0.41, P=0.002 \); fibrous plaque: \( r=0.54, P<0.001 \); calcified plaque: \( r=0.59, P<0.001 \); total plaque: \( r=0.62, P<0.001 \)). We also assessed VH-IVUS and CT Plaque Maps against coregistered histology in 72 (VH-IVUS) and 87 (CT) segments from 8 postmortem coronary arteries. The diagnostic accuracy of CT to detect calcified plaque (83% versus 92%), necrotic core (80% versus 65%), and fibroatheroma (80% versus 79%) was comparable with VH-IVUS. However, although VH-IVUS could identify thin-cap fibroatheromas (TCFA) with a diagnostic accuracy of between 74% and 82% (depending on the TCFA definition used), the spatial resolution of CT prevented direct identification of TCFA.

Conclusions—CT-derived Plaque Maps based on contrast-adjusted attenuation ranges can define individual plaque components with a similar accuracy to VH-IVUS ex vivo. However, coronary CT Plaque Maps could not reliably classify plaques and identify TCFA, such that high-risk plaques may be misclassified or overlooked. (Circ Cardiovasc Imaging. 2013;6:655-664.)

Key Words: atherosclerosis ■ imaging

Postmortem studies have revealed that atherosclerotic plaques associated with coronary thrombosis, sudden death, and plaque rupture typically have less fibrous tissue, a thinner fibrous cap, and a larger necrotic core than stable plaques. In particular, the plaque type most frequently associated with rupture is the thin-cap fibroatheroma (TCFA), where a cap of 65 μm separates a large necrotic core from the lumen. Although these features potentially allow TCFA to be identified in vivo, it requires an imaging modality that can discriminate reliably between plaque components (fibrous tissue, necrotic core) and identify their location within the plaque with a spatial resolution that can identify a thin cap.

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Virtual histology intravascular ultrasound (VH-IVUS) is an ultrasound-based technique that analyzes ultrasound backscatter to characterize atherosclerotic plaques into necrotic core, fibrous tissue, fibro-fatty tissue, and dense calcium. VH-IVUS has also been used to classify plaques into virtual histology versions of traditional histological definitions. Some studies report that VH-IVUS–identified TCFA (VHTCFAs) are increased in acute coronary syndrome (ACS) versus stable angina patients, and recent prospective studies have shown that VHTCFAs have a higher risk of major adverse cardiac events than other types of...
plaque components have been validated against human postmortem histology, human coronary plaque classification and the different VHTCFA definitions have not.

Although VH-IVUS is used extensively for research, noninvasive identification of vulnerable lesions may be preferable for routine clinical use, particularly in patients who are not undergoing intervention. Computed tomography (CT) has emerged over recent years as a safe and reliable imaging technique to detect atherosclerotic plaque size and luminal stenosis in coronary arteries, and can provide limited information on plaque composition. For example, calcified plaque can be discriminated from other components due to its much higher attenuation. In addition, certain CT features such as the signet ring sign, spotty calcification, low attenuation plaque, and positive remodeling are increased in patients presenting with ACS versus stable angina, and low attenuation segments with positive remodeling were associated with highest risk of ACS in prospective studies. In contrast, discrimination of other plaque constituents is not well validated. For example, significant overlap in attenuation of necrotic core and fibrous plaque has limited previous studies using grey-scale IVUS to define the CT attenuation of plaque components. Furthermore, although 64-slice CT can identify large grey-scale IVUS-defined lipid pools with a sensitivity of 70%, grey-scale IVUS does not detect necrotic core reliably, making it a poor modality with which to compare CT. The effect of contrast enhancement on plaque attenuation has also not been addressed.

We assessed the ability of dual-source 64-slice CT to distinguish VH-IVUS–defined plaque components based on their x-ray attenuation. The attenuation values were then used to create color-coded Plaque Maps that used novel ratios of plaque to contrast attenuation to automatically adjust for interpatient variation in contrast intensity and reduce attenuation overlap. Plaque Maps were tested for diagnostic accuracy in a separate in vivo validation patient cohort against VH-IVUS, and ex vivo coronary arteries against the gold standard of human postmortem histology. We find that CT can identify plaque components such as fibrous tissue and necrotic core with similar accuracy to VH-IVUS ex vivo; however, CT could not accurately classify plaques and identify TCFAs.

Methods

Clinical Study

Virtual Histology Intravascular Ultrasound

Following informed consent and approval from the Cambridgeshire Regional Ethics Committee patients with stable angina or ACS undergoing percutaneous coronary intervention underwent both VH-IVUS and CT coronary angiography. VH-IVUS was acquired using Eagle-Eye Gold catheters and motorized pullback as described previously. Plaques with VH-IVUS–defined plaque burden >40% were chosen for coregistration with CT. Coregistered cross-sectional frames from each plaque were selected if they contained VH-IVUS–defined homogenous fibrous plaque, or large areas of confluent necrotic core, or calcified plaque to determine the CT attenuation of VH-IVUS–defined plaque components. The proximal 30 mm of both the culprit and larger of the nonculprit coronary arteries was used to provide geometric and compositional data plaque for comparison of VH-IVUS with CT-acquired data.

Computed Tomography

All patients underwent a retrospectively gated CT scan with ECG-dependent tube current modulation using a Somatom Definition 64-slice dual-source CT (Siemens, Germany; pitch, 0.20–0.48; collimation, 32×2 mm×0.6 mm; tube voltage, 120 kV, and current, 360 mA). Intravenous contrast (Niopam 370; Bracco Ltd, United Kingdom) was used in a triphasic injection protocol following a 20-mL timing bolus to assess circulation time. This gave a spatial resolution of 0.4×0.4×0.4 mm³ and temporal resolution of 83 ms. All CT analysis was performed using Circulation III software (Siemens, Germany). Curved multiplanar reconstructions of the coronary arteries were compared with longitudinal reconstructed IVUS data sets, an approach used in previous studies.

To ensure accurate plaque coregistration, plaque locations were triangulated using fiduciary points, including proximal reference measurements from coronary ostia and distal reference measurements from landmark side branches or vessel bifurcations. Cross-sectional CT images were matched with corresponding VH-IVUS frames and multiple regions of interests (ROIs) sampled on CT images in areas preclassified by VH-IVUS as necrotic core, fibrous plaque, calcified plaque, or lumen, resulting in CT attenuation values for each plaque component within these ROIs (expressed in Hounsfield units, HU). Intraobserver variability was calculated by repeating measurements 1 week apart (Figure I in the online-only Data Supplement).

The mean attenuation of luminal contrast for each plaque was calculated by measuring luminal attenuation proximal and distal to each plaque. The ratio of plaque attenuation to its corresponding contrast attenuation was calculated for all sampled areas and used to assign ranges of plaque/contrast attenuation ratios to each plaque component. Curved multiplanar reconstructions of the coronary artery segments that corresponded to those chosen for VH-IVUS analysis were created and used to produce Plaque Maps using both fixed attenuation ranges of plaque components and ranges of plaque/contrast attenuation ratios. These gave quantitative measurements of vessel geometry and plaque composition that were compared with VH-IVUS–derived values to calculate the correlation between the 2 imaging modalities.

Postmortem Specimen Preparation and Histology

Postmortem hearts were used with informed consent from relatives and approval from the Cambridgeshire Regional Ethics Committee. Patient details are provided in the online-only Data Supplement. The left anterior descending artery was dissected <24 hours after death within <40 to 50 mm of surrounding tissue to maintain its structural integrity. The vessel was embedded in wax for mechanical support and the left main stem ostium and distal end of the vessel cannulated. The vessel was then submerged and pressure-perfused with phosphate-buffered saline (at 37°C) at 100 mm Hg before VH-IVUS (see the online-only Data Supplement) and CT image acquisition. Arteries were imaged using tube voltage 120 kV and current 360 mA. Intravenous contrast (Niopam 370) was injected at a perfusion pressure of 100 mm Hg following dilution with phosphate-buffered saline to achieve luminal attenuation comparable with clinical scans (261–465 HU). Following imaging, arteries were processed for histology and classified (Figure II in the online-only Data Supplement).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5.00 (GraphPad Software) and R software (R Foundation for Statistical Computing, Austria; http://www.R-project.org/). Continuous variables are presented as means with standard deviations if normally distributed and medians with interquartile ranges (Q1–Q3) if not. Differences in plaque component attenuation were assessed using a linear mixed-effects model (to account...
for multiple attenuation values per patient) fitted at the level of the plaque to the attenuation outcome with patient as a random effect and assuming a compound symmetry structure. Plaque component was included in the model as an explanatory factor variable with 4 levels (necrotic core, fibrous plaque, lumen, and calcified plaque) with necrotic core as the reference category. Assessment of the imaging modalities’ ability to perform plaque classification was expressed using point estimates of sensitivity, specificity, and diagnostic accuracy with each plaque considered an independent data point. Agreement between paired data (VH-IVUS and CT) was assessed using Bland–Altman plots and differences in mean volumes between CT and VH-IVUS using paired t tests. Correlation between measurements was expressed as the Pearson correlation coefficient (r).

Results

Determination of CT Attenuation of Plaque Components

To examine the ability of coronary CT to identify plaque composition, we first imaged 57 patients with 3-vessel VH-IVUS followed by coronary CT and coregistered 108 frames from 108 plaques (Figure 1). We sampled 855 ROIs on CT images that corresponded with defined tissue types on VH-IVUS (236 necrotic core, 215 fibrous plaque, 260 calcified plaque, 144 lumen). Median HU values with Q1 to Q3 range for each component were: necrotic core, 39 (−35 to 159); fibrous plaque, 106 (0–294); arterial lumen, 362 (246–545); calcified plaque, 770 (358–1587; Figure 2A). Using a linear mixed-effects model, there were significant differences in attenuation between the different components. Relative to necrotic core, the mean difference (95% confidence interval) in attenuation was: fibrous plaque, 78 HU (41–115); lumen, 322 HU (281–363); and calcified plaque, 741 HU (698–783; all P≤0.01).

Defining Plaque Components With Plaque/Contrast Attenuation Ratios

Although different plaque components showed different HU ranges, plaque attenuation is affected by patient parameters such as differing body habitus. Plaque attenuation is also affected by differences in contrast attenuation in the adjacent lumen in ex vivo studies. We, therefore, corrected HU values for each component by comparison with contrast attenuation in the lumen across plaques. Mean attenuation was calculated in the corresponding lumen of the 108 coregistered plaques and used to create 108 plaque attenuation/contrast ratios (35 necrotic core, 34 fibrous plaque, 39 calcified plaque). Median ratios with Q1 to Q3 range were: necrotic core, 0.1013 (0.0220–0.2521); fibrous plaque, 0.2843 (0.1431–0.4746); and calcified plaque, 1.942 (1.281–3.191).

Figure 1. CT attenuation sampling of virtual histology intravascular ultrasound (VH-IVUS)–defined plaque. Curved multiplanar reformatted images of coronary arteries (left) with cross-sectional images (middle) taken at site of white arrow. VH-IVUS–defined plaque components (right) were used for sampling CT attenuation of (A) fibrous plaque, (B) calcified plaque, and (C) necrotic core.

Figure 2. CT attenuation of different plaque tissue types. Box and whisker plots of CT attenuation (Hounsfield units; A,) or plaque/contrast attenuation ratios (B) of the virtual histology intravascular ultrasound (VH-IVUS)–defined plaque tissue types and lumen. Data are median (Q1–Q3), showing statistically significant differences between groups (linear mixed-effects model; *P<0.01).
Ratios were statistically significantly different from each other \((P<0.01)\) using a linear mixed-effects model (Figure 2B).

### Creation of CT Plaque Maps

Plaque Maps were created using the Circulation III software (Siemens, Germany). Four ranges of attenuation were set manually, representing 4 different color-coded arterial components. Attenuation ranges were assigned as necrotic core (dark green), fibrous plaque (light green), lumen (orange), and calcified plaque (pink) based both on HU values from coregistered VH-IVUS areas and contrast/plaque attenuation ratios. Plaque Map ranges were set using the halfway point between upper (95th percentile) of values obtained for one component and lower (5th percentile) of values obtained for the adjacent component. This gave values free of overlap between all components, except necrotic core and fibrous plaque. The upper 95th percentile of necrotic core (94 HU) and lower 5th percentile of fibrous plaque (33 HU) gave a halfway cutoff of 65 HU. Although this cutoff resulted in some overlap in Plaque Map ranges, it still separated the upper 75th percentile (Q3) of necrotic core attenuation values (58 HU) from the lower 25th percentile (Q1) of fibrous plaque (77 HU).

We also created Plaque Map ranges from plaque/contrast attenuation ratios, again using the 95th and 5th percentile values. There was some overlap between upper 95th plaque/contrast attenuation ratio for necrotic core (0.2463) and lower 5th percentile for fibrous plaque (0.1909), with a halfway cutoff of 0.197. The ratio values were converted into attenuation ranges by multiplying by the attenuation of contrast sampled in the arterial segment in which the Plaque Map is created, giving ranges tailored to individual patients. Ranges of fixed attenuation and plaque/contrast attenuation ratio used to create Plaque Maps are listed in Table 1.

### Assessment of Plaque Maps: In Vivo Correlation Between CT and VH-IVUS

To assess how well Plaque Maps generated using plaque/contrast attenuation ratios were able to quantify VH-IVUS–defined plaque components in vivo, we studied a validation cohort of 47 patients undergoing both VH-IVUS and CT, with volumetric analysis of coregistered 30-mm proximal coronary artery segments. This cohort included 23 patients with stable angina (12 culprit artery and 22 nonculprit segments) and 24 patients with ACS (14 culprit artery and 24 nonculprit segments) giving 72 segments (2160 mm). Volumes of plaque components were averaged per patient before analysis to avoid using multiple measures per patient.

Plaque volumes assessed by VH-IVUS and CT Plaque Maps using plaque/contrast attenuation ratios were highly correlated \((P<0.001)\) for necrotic core \((r=0.41)\), fibrous plaque \((r=0.54)\), calcified plaque \((r=0.59)\), total plaque \((r=0.62)\), lumen \((r=0.77)\), and vessel \((r=0.80)\; \text{Table 2;} \text{Figure 3})

The mean volumes (±SD) estimated by Plaque Maps created with plaque/contrast attenuation ratios were similar to those estimated by VH-IVUS for calcified plaque (15.8 mm3±17.0 versus 15.4 mm3±13.2) and vessel lumen (283 mm3±94.6 versus 273 mm3±97.5). However, compared with VH-IVUS, CT volumes were higher for necrotic core (121 mm3±53.2 versus 33.4 mm3±21.6), fibrous plaque (163 mm3±35.7 versus 105 mm3±44.4), total plaque (300 mm3±86.3 versus 251 mm3±73.5), and vessel (646 mm3±177 versus 523 mm3±137; Table 2). Bland–Altman plots showing the limits of agreement for 95% of the difference between CT and VH-IVUS are shown in Figure 4.

### Assessment of Plaque Maps: Ex Vivo Correlation With Postmortem Histology

Clearly, comparison with VH-IVUS to determine accuracy of CT depends in part on the accuracy of VH-IVUS itself to identify plaque components. To examine whether the CT

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**Table 1. Median Attenuation Values (HU) and Contrast/Plaque Attenuation Ratios Obtained for Each Plaque Component With Interquartile Range (Q1–Q3), 5th to 95th Percentile, and the Derived Ranges for Each Component Used to Create Plaque Maps**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Median Value</th>
<th>25th to 75th Percentile</th>
<th>5th to 95th Percentile</th>
<th>Range for Plaque Map</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrotic core</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attenuation values, HU</td>
<td>39</td>
<td>21–58</td>
<td>–1 to 94</td>
<td>–1 to 64</td>
</tr>
<tr>
<td>Contrast/plaque attenuation ratio</td>
<td>0.1013</td>
<td>0.0737–0.1360</td>
<td>0.0311–0.2463</td>
<td>&lt;0.197</td>
</tr>
<tr>
<td>Fibrous plaque</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attenuation values, HU</td>
<td>106</td>
<td>77–146</td>
<td>33–249</td>
<td>65–260</td>
</tr>
<tr>
<td>Contrast/plaque attenuation ratio</td>
<td>0.2843</td>
<td>0.1909–0.3814</td>
<td>0.1480–0.4696</td>
<td>0.197–0.470</td>
</tr>
<tr>
<td>Calcified plaque</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attenuation values, HU</td>
<td>770</td>
<td>641–885</td>
<td>471–1246</td>
<td>&gt;465</td>
</tr>
<tr>
<td>Contrast/plaque attenuation ratio</td>
<td>1.942</td>
<td>1.568–2.182</td>
<td>1.295–0.099</td>
<td>&gt;1.295</td>
</tr>
<tr>
<td>Lumen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attenuation values, HU</td>
<td>362</td>
<td>329–396</td>
<td>270–458</td>
<td>261–465</td>
</tr>
</tbody>
</table>

HU indicates Hounsfield units.
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attenuation ranges derived in vivo truly identified the appropriate tissue type, we undertook combined ex vivo VH-IVUS, CT with creation of Plaque Maps using our defined attenuation ranges, and histology (Figure 5). We analyzed 87 segments of ≈400 µm in length in 8 postmortem arteries. Histological analysis revealed that 61 of 87 (70%) segments contained significant atherosclerotic plaque, 33 of 87 (38%) contained necrotic core, and 54 of 87 (62%) contained calcified plaque. VH-IVUS analysis was possible in 72 segments and was excellent at determining the presence of significant atherosclerotic plaque, detecting it in 56 of 56 segments and excluding it correctly in 15 of 16 segments, with a sensitivity, specificity, and diagnostic accuracy of 100%, 94%, and 99%, respectively. VH-IVUS was similarly successful in distinguishing the presence of calcification (sensitivity 96%, specificity 90%, diagnostic accuracy 92%). The sensitivity to detect necrotic core was 100%; however, the specificity was 40% (diagnostic accuracy 65%).

Table 2. Mean Volumes and Correlations Between Measurements Obtained Using CT-Derived Plaque/Contrast Attenuation Ratios and VH-IVUS

<table>
<thead>
<tr>
<th></th>
<th>CT, Mean±SD</th>
<th>VH-IVUS, Mean±SD</th>
<th>P Value (t test)</th>
<th>Mean Difference</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Absolute</td>
<td>%</td>
</tr>
<tr>
<td>NC, mm³</td>
<td>121 (53.2)</td>
<td>33.4 (21.6)</td>
<td>&lt;0.001</td>
<td>87.1</td>
<td>111</td>
</tr>
<tr>
<td>NC, %</td>
<td>38.2 (8.9)</td>
<td>20.5 (9.0)</td>
<td>&lt;0.001</td>
<td>17.8</td>
<td>63.2</td>
</tr>
<tr>
<td>Fi, mm³</td>
<td>164 (35.7)</td>
<td>105 (44.4)</td>
<td>&lt;0.001</td>
<td>58.6</td>
<td>48.5</td>
</tr>
<tr>
<td>Fi, %</td>
<td>53.6 (10.2)</td>
<td>69.7 (13.6)</td>
<td>&lt;0.001</td>
<td>−16.1</td>
<td>−25.5</td>
</tr>
<tr>
<td>Ca, mm³</td>
<td>15.8 (17.0)</td>
<td>15.4 (13.2)</td>
<td>0.86</td>
<td>0.4</td>
<td>−54.7</td>
</tr>
<tr>
<td>Ca, %</td>
<td>4.6 (4.8)</td>
<td>33.4 (21.6)</td>
<td>&lt;0.001</td>
<td>−28.8</td>
<td>−154</td>
</tr>
<tr>
<td>PL, mm³</td>
<td>300 (86.3)</td>
<td>251 (73.5)</td>
<td>&lt;0.001</td>
<td>49.3</td>
<td>17.6</td>
</tr>
<tr>
<td>Lu, mm³</td>
<td>283 (94.6)</td>
<td>273 (97.5)</td>
<td>0.30</td>
<td>9.8</td>
<td>3.7</td>
</tr>
<tr>
<td>Ve, mm³</td>
<td>646 (177)</td>
<td>523 (137)</td>
<td>&lt;0.001</td>
<td>123</td>
<td>20.7</td>
</tr>
</tbody>
</table>

Ca indicates calcified plaque; Fi, fibrous plaque; Lu, total lumen volume; NC, necrotic core; PL, total plaque volume; and Ve, total vessel volume.

Figure 3. Correlations between CT and virtual histology intravascular ultrasound (VH-IVUS) measurements. Scatter plots demonstrating significant correlations (P<0.01) between CT-generated Plaque Maps using plaque/contrast ratios and VH-IVUS for volumes of plaque components, total plaque area, luminal area, and total vessel area.
CT (performed in all 87 segments) detected significant atherosclerosis with a sensitivity, specificity, and diagnostic accuracy of 89%, 58%, and 79%. The lower specificity compared with VH-IVUS occurred in scans with lower than average luminal contrast intensity, in which contrast attenuation in the lumen periphery entered the range defined as fibrous plaque, giving a false-positive (Figure III in the online-only Data Supplement). Plaque Maps created from plaque/contrast attenuation ratios detected significant plaque with a sensitivity, specificity, and diagnostic accuracy of 90%, 92%, and 91%. Plaque/contrast attenuation ratios automatically adjust for lower luminal contrast intensity, reducing the number of false-positive results (Figure III in the online-only Data Supplement).

The sensitivity, specificity, and diagnostic accuracy of Plaque Maps derived using fixed attenuation ranges for calcified plaque was 78%, 85%, and 80%, and for necrotic core was 55%, 93%, and 78%. Plaque/contrast attenuation resulted in only a marginal improvement (sensitivity, specificity, and diagnostic accuracy of calcified plaque was 80%, 88%, and 83% and for necrotic core was 55%, 96%, and 80%). However, if only necrotic core ≥2 mm² on histology was considered, the sensitivity increased to 75%.

We also examined the ability of both CT Plaque Maps and VH-IVUS to classify plaques compared with histology (Table 3). VH-IVUS could identify all plaque types with an accuracy of ≥74%, including fibroatheromas (Figure II in the online-only Data Supplement). Plaque Maps created with plaque/contrast attenuation ratios identified histologically determined fibroatheromas (plaque with necrotic core) with a sensitivity, specificity, and diagnostic accuracy of 55%, 96%, and 80%. Overall diagnostic accuracy of CT was similar for most plaque types compared with VH-IVUS (Table 3). The important exception to this was TCFA, which could not be identified by CT compared with histology. VH-TCFAs are defined by necrotic core in contact with lumen; however, a similar definition still did not allow identification of TCFA by CT because of partial volume artefacts from luminal contrast (Figure IV in the online-only Data Supplement).

**Discussion**

Although VH-IVUS has been shown to identify plaques at increased risk of major adverse cardiac events in prospective studies, noninvasive imaging modalities such as CT might be preferable to VH-IVUS if they possess similar abilities to detect plaque composition and classify plaque.
We find that different plaque components show significant differences in HU attenuation, an essential feature if they are to be distinguished successfully by CT. Attenuation ranges could be demarcated between calcified plaque and lumen, and fibrous plaque and lumen, which meant that each component contained attenuation values between the 5th and 95th percentiles of all those obtained, allowing maximum discrimination. Although there was an overlap between the 5th and 95th percentiles of attenuation values of necrotic core and fibrous plaque, the limits chosen allowed each range to contain all attenuation values within the 25th to 75th percentiles for each component. We also defined plaque components based on the ratio of attenuation of plaque to luminal contrast rather than of plaque alone. Ranges of plaque/contrast attenuation ratios were also significantly different for each plaque component, and volumes of each component identified using these ratios were significantly correlated with volumes identified by VH-IVUS in vivo. Finally, attenuation ranges and plaque/contrast attenuation ratios showed similar sensitivity, specificity, and accuracy to detect plaque components as VH-IVUS when compared against histology.

Although ex vivo validation of CT-derived Plaque Maps is important, in vivo accuracy is critical and cannot be automatically inferred from ex vivo studies. Patient body habitus and heart rate both affect in vivo resolution of CT. Plaque Maps created with fixed attenuation ranges detected significant atherosclerotic plaque and calcified plaque with diagnostic accuracies of 79% and 80%, respectively. The accuracy to detect necrotic core was lower, but still reasonable (78%); however, the sensitivity to detect necrotic core was 55% and reflects the spatial resolution of CT that prevents detection of small areas of necrotic core. However, detection of small areas of necrotic core may not be clinically important, given that ruptured plaques on postmortem examination typically have necrotic core areas >2 mm². Repeat analysis using necrotic cores of ≥2 mm² gave a sensitivity of CT of 75%.

We repeated this analysis with Plaque Maps constructed from plaque/contrast attenuation ratios, which automatically adjust to match variations in contrast attenuation between patients. To our knowledge, this is a novel way of defining coronary plaque components using CT. These ratios led to an overall increase in diagnostic accuracy of plaque components compared with fixed ranges when tested against both in vivo VH-IVUS and postmortem histology. The improvement of attenuation ratios over fixed ranges was seen particularly in the specificity to detect significant atherosclerotic plaque (92% versus 58%) and thus reduced the potential for false-positive diagnosis of significant lesions.

We have shown that it is possible to obtain measurements on coronary vessel geometry and plaque composition using CT that show significant correlation for all measurements compared with VH-IVUS. These correlations were also stronger if plaque components were defined using contrast/plaque attenuation ratios. Given the strength of these correlations, it might be possible for serial CT analysis of coronary plaque burden and composition to provide a noninvasive alternative to serial VH-IVUS in clinical trials and studies on the natural history of atherosclerotic plaques, potentially making these studies simpler, safer, and cheaper. However, large percentage differences and limitations of agreement found on the Bland–Altman analysis mean that absolute volumes obtained by CT and VH-IVUS on individual coronary segments should not be compared. The greatest percentage difference between CT and VH-IVUS was seen for necrotic core volume, with CT detecting substantially more necrotic core than VH-IVUS. The correlation between CT and VH-IVUS for necrotic core volume was also the weakest of all plaque components (r=0.41). Given the overlap between their attenuation ranges, CT may misclassify some fibrous plaque as necrotic core. Another possible explanation may be that VH-IVUS classifies necrotic core based on a heterogeneous collection of components including lipid, type.
cholesterol clefts, and microcalcification. Plaque containing large amounts of lipid may be classified by VH-IVUS as fibro-fatty tissue but have low attenuation, and be classified as necrotic core on CT. Contrary to expectations, CT underestimated calcified plaque compared with VH-IVUS by 55% on average. CT would be expected to overestimate calcified plaque due to partial voluming (although increased spatial resolution of modern scanners has decreased this effect). However, VH-IVUS is sensitive at detecting small amounts of calcification that may be beyond the spatial resolution of CT. Although the effect of these volumes of calcified plaque were small in absolute terms (mean volumes of calcified plaque on CT and VH-IVUS were 15.8 versus 15.4 mm$^3$; $P=0.86$), comparing even small volumes detected on VH-IVUS with none detected by CT will lead to large percentage differences.

Comparison between VH-IVUS and CT in vivo can introduce differences due to the limitations of both techniques. In addition, VH-IVUS is not universally accepted as being able to determine plaque composition, and coronary plaque classification by VH-IVUS has not been validated ex vivo against postmortem whole artery histology. We, therefore, assessed both VH-IVUS and CT tissue characterization and plaque classification against postmortem human coronary arteries and tested the accuracy of 2 different VH-TCFA definitions in use. VH-IVUS diagnostic accuracy was 79% to classify all fibroatheroma. VH-IVUS had 87% sensitivity to detect fibroatheroma, whereas the sensitivity to detect TCFA varied from 14% to 71%, depending on the definition used.

The diagnostic accuracy of ex vivo CT to detect fibroatheroma was comparable with VH-IVUS (77%–80%). In contrast, although VH-IVUS reliably detected TCFAs, CT could not detect TCFAs, in part because 65 μm or even 200 μm is beyond its current spatial resolution. It was also not possible to visualize necrotic core directly adjacent to lumen on CT Plaque Maps (as used to define TCFA with VH-IVUS). VH-IVUS thus remains the only modality with both histological evidence that it reliably identifies plaque components and prospective clinical natural history outcome data of these plaques.

**Limitations**

Given the current limit of spatial resolution, this study was designed to assess how well CT could identify plaque components and classify plaques compared with VH-IVUS in vivo and both VH-IVUS and histology ex vivo. Although CT identified plaque components with similar accuracies to VH-IVUS ex vivo, our analysis has several limitations. First, the validity of CT attenuation values derived from VH-IVUS depends on the ability of VH-IVUS itself to identify plaque composition. In vivo, VH-IVUS diagnostic accuracy for necrotic core within heavily calcified plaques is limited due to artifact related to the processing algorithm. We mitigated this limitation when defining the attenuation profile of fibrous plaque and necrotic core by

<table>
<thead>
<tr>
<th>Histologically Defined Plaque</th>
<th>Correctly Classified by CT Plaque Map</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Diagnostic Accuracy</th>
</tr>
</thead>
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<tr>
<td>All fibroatheroma</td>
<td>18/33</td>
<td>55%</td>
<td>93%</td>
<td>78%</td>
</tr>
<tr>
<td>Fixed attenuation ranges</td>
<td>18/33</td>
<td>55%</td>
<td>96%</td>
<td>80%</td>
</tr>
<tr>
<td>Contrast/plaque attenuation ratio</td>
<td>18/33</td>
<td>55%</td>
<td>96%</td>
<td>80%</td>
</tr>
<tr>
<td>Calified fibroatheroma</td>
<td>12/27</td>
<td>44%</td>
<td>95%</td>
<td>79%</td>
</tr>
<tr>
<td>Fixed attenuation ranges</td>
<td>12/27</td>
<td>44%</td>
<td>97%</td>
<td>80%</td>
</tr>
<tr>
<td>Contrast/plaque attenuation ratio</td>
<td>12/27</td>
<td>44%</td>
<td>97%</td>
<td>80%</td>
</tr>
<tr>
<td>Fibrocalcific plaque</td>
<td>20/26</td>
<td>77%</td>
<td>79%</td>
<td>78%</td>
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<tr>
<td>Fixed attenuation ranges</td>
<td>20/26</td>
<td>77%</td>
<td>79%</td>
<td>78%</td>
</tr>
<tr>
<td>Contrast/plaque attenuation ratio</td>
<td>20/26</td>
<td>77%</td>
<td>79%</td>
<td>78%</td>
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<tr>
<td>All TCFA (cap &lt;65 μm)</td>
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<tr>
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<tr>
<td>Contrast/plaque attenuation ratio</td>
<td>0/7</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Table 3. Sensitivity, Specificity, and Diagnostic Accuracy of Ex Vivo CT and VH-IVUS Plaque Classification Compared With Histology**

N/A indicates not available; VH-IVUS, virtual histology intravascular ultrasound; VH-TFCFA, TCFA (thin-cap fibroatheromas) requiring any contact of necrotic core and lumen; and TCFA>36°; TCFA requiring contact of necrotic core with lumen for more than 36°.
choosing coregistered VH-IVUS frames that did not contain significant dense calcification. Second, VH-IVUS and CT validation ex vivo was performed on segments from 8 coronary arteries, a relatively small number. However, we obtained 87 segments coregistered with histology that provided robust statistical analysis. Third, only left anterior descending arteries were used, but we consider it likely that VH-IVUS and CT would perform similarly in other vessels. Fourth, ex vivo validation was performed in optimum scanning conditions (stationary coronary arteries and minimal surrounding tissue) and not in a phantom where in vivo conditions might be better simulated. Diagnostic accuracy may be affected by temporal resolution with high heart rates or in larger patients where the image may be degraded by noise. Fifth, despite identifying HU ranges that gave the most accurate discrimination between tissue types based on CT attenuation, there was still some overlap between necrotic core and fibrous tissue, and detection of thin fibrous caps remains beyond the spatial resolution of CT. This is because structures smaller than its spatial resolution (400 μm) are represented by their average attenuation. As attenuation of luminal contrast is greater than of necrotic core, average attenuation at the border can lead to a misclassification as higher attenuation, that is, fibrous plaque (an artifact known as partial voluming).

Conclusions
We have shown that CT Plaque Maps defined by HU ranges against VH-IVUS identify plaque components with comparable diagnostic accuracy to VH-IVUS when validated against postmortem histology. Accuracy was further improved if components were defined by plaque/contrast attenuation ratios to adjust for interpatient variation in contrast intensity. Thus, CT-derived Plaque Maps may be a valuable tool for studies aimed at identifying changes in atherosclerotic plaque composition. However, the current spatial resolution of 64-slice CT means that Plaque Maps could not identify TCFA, which remains reliant on intracoronary imaging.

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Disclosures
None.

References


**CLINICAL PERSPECTIVE**

Myocardial infarction is most frequently caused by rupture of a specific type of plaque, the thin-cap fibroatheroma (TCFA); identification of these plaques before rupture is therefore of considerable clinical significance. Although invasive imaging, for example, virtual histology intravascular ultrasound (VH-IVUS), can identify TCFA, routine clinical use (eg, for screening) would need to be noninvasive. We examined the ability of coronary CT to identify plaque composition and plaque type compared with both VH-IVUS and postmortem histology. We identified attenuation ranges for CT that reliably identify tissue composition in plaques and showed that there is significant correlation between plaque component volumes determined by CT and VH-IVUS, although absolute volumes differ. We found that VH-IVUS identifies all different plaque types compared with histology with diagnostic accuracies >70%. In contrast, although the ex vivo diagnostic accuracy of CT to detect calcified plaque, necrotic core, and fibroatheroma was comparable to VH-IVUS, CT could not identify TCFA. Our study shows that plaque size and composition can be detected with good accuracy by CT. CT using defined contrast/attenuation ratios could, therefore, be used to monitor plaque progression/regression and changes in composition in therapeutic or natural history studies. However, the resolution of CT means that it cannot identify TCFA, and currently this requires invasive imaging such as VH-IVUS.
Atherosclerotic Plaque Composition and Classification Identified by Coronary Computed Tomography – Generated Plaque Maps Compared With Virtual Histology Intravascular Ultrasound and Histology

Daniel R. Obaid, Patrick A. Calvert, Deepa Gopalan, Richard A. Parker, Stephen P. Hoole, Nick E.J. West, Martin Goddard, James H.F. Rudd and Martin R. Bennett

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Atherosclerotic plaque composition and classification identified by coronary CT:
Assessment of CT-generated plaque maps compared with VH-IVUS and histology

SUPPLEMENTAL MATERIAL
Supplemental Methods
Reproducibility of CT-derived plaque component attenuation

To determine the intra-observer reproducibility of CT attenuation for each component, 152 regions of interest were sampled in 19 cross-sectional images (co-registered with VH-IVUS) taken from 19 coronary arteries from 7 patients, and repeated 1 week later. Mean attenuation of the regions of interest from each VH-IVUS defined component were similar between samples: Necrotic core = 46.3±30.1 vs. 42.4±50.0 (p = 0.79), fibrous plaque = 119.1±50.1 vs. 151.1±51.5 (p=0.09), lumen = 313.7±70.8 vs. 303.0±71.2 (p=0.43) and calcified plaque = 505.8±99.8 vs. 516.5±109.4 (p=0.57). Intra-observer variability differed depending on the plaque component being sampled, with 97% of values within 10% confidence intervals for calcified plaque, 90% for lumen, 62% for fibrous plaque and 56% for necrotic core (Supplemental Figure 1).

Patients for post mortem analysis
Ten human post-mortem coronary arteries were studied. Donor subjects had a mean patient age of 70.5±9 years, 40% of patients were male and in 40% the cause of death was directly related to coronary artery disease. VH-IVUS acquisition was possible in 8/10 arteries yielding 78 400µm long segments. 6 segments contained a coronary stent and were excluded from the analysis leaving 72 VH-IVUS segments co-registered with histology. 19/72 segments (26%) had no significant atherosclerosis, containing either adaptive intimal
thickening only (n=15) or intimal xanthoma (n=4). Calcification was found in 51/72 (72%) of segments and necrotic core in 30/72 (41%).

**Histology**

Following imaging, arteries were fixed with formalin (after decalcification if required). The external surface of the artery was marked at 1 cm intervals to aid co-registration. Each 1 cm block of the coronary artery was cut into 400 µm length segments, which were processed for histology and sectioned at 5 µm intervals. Plaque composition was determined using haematoxylin and eosin (H+E) and Movat’s Pentachrome stains. Digital histology images were acquired and analysed by an expert Histopathologist (MG) with over 20 years experience to identify areas composed of (a) confluent necrotic core, (b) fibrous tissue and (c) calcified plaque. If a necrotic core was present then the minimum diameter of any overlying fibrous cap was measured. Each plaque segment was classified according to the following accepted histological definitions¹.

**Non-significant atherosclerotic plaque:**

1. Normal artery.
2. Adaptive intimal thickening (accumulation of intimal smooth muscle cells without lipid or foam cells).
3. Intimal xanthoma (accumulation of foam cells with absence of a necrotic core or fibrous cap).

**Significant atherosclerotic plaque:**

1. Fibrous plaque (fibrotic lesion with minimal lipid).
2. Pathological intimal thickening (accumulation of smooth muscle cells and extra-cellular lipid pools).
3. Fibrocalcific plaque (collagen-rich plaque with large area of calcification, without lipid pool).
4. Fibroatheroma (presence of well-defined lipid-rich necrotic core, calcified fibroatheroma if significant calcification also present).
5. Thin-capped fibroatheroma (fibroatheroma with separation of necrotic core from lumen by a fibrous cap of 65 µm or less).

Post-mortem VH-IVUS

An Eagle-Eye Gold catheter (Volcano Corporation, Rancho Cordova, California, USA) was inserted over a guide wire into each post-mortem artery. An automated pullback was then performed during which VH-IVUS images were acquired. The longitudinal pullback data was separated into segments approximately 400 µm in length, matching segments used for histological analysis. Each segment contained a median number of 11 (range 6-17) VH-IVUS frames that were analysed sequentially. The presence of significant plaque (plaque burden >40%), calcified plaque (>10% dense calcium) or necrotic core (>10%) in 3 consecutive frames) was recorded for comparison with post-mortem histology. Each segment was also classified according to accepted VH-IVUS definitions\(^2\).

1. No significant plaque (plaque burden <40%).
2. Fibrocalcific (significant (>10%) calcified plaque and <10% necrotic core).
3. Fibroatheroma (presence of significant (>10%) necrotic core in 3 consecutive frames).
4. Calcified fibroatheroma (as above with significant (>10%) calcified plaque).
5. Thin-cap fibroatheroma (significant (>10%) necrotic core with no overlying fibrous cap for 3 consecutive frames). The most common definition of a VH-IVUS-defined TCFA (VH-
TCFA) requires any necrotic core contact with the lumen, but a recent consensus document has proposed a definition requiring 36° of necrotic core / luminal contact. Both definitions were examined.

6. Calcified thin-cap fibroatheroma (as above with significant (>10%) calcified plaque).
Supplemental Figure 1. Bland–Altman Analysis of the Intra-observer variability in attenuation of regions of interest placed in plaque components 1 week apart
Mean attenuation is plotted on the x-axis against the % difference between the 2 samples on the y-axis. Acceptable variation was considered to be within 10% and these confidence intervals are plotted on the y-axis.
Supplemental Figure 2. VH-IVUS validation by post-mortem histology
Co-registered histology (left) and VH-IVUS (right) for (A) Non-significant atherosclerosis (adaptive intimal thickening), (B) Fibroatheroma (VH-IVUS: red-necrotic core, green-fibrous plaque), (C) Calcified fibroatheroma (VH-IVUS: white-calcified plaque), (D) Thin-cap fibroatheroma. Black arrow points to area of focal fibrous cap thinning with rupture. White arrow points to small area of necrotic core (red) / luminal contact.
Supplemental Figure 3. Plaque / contrast attenuation ratios reduce false positive detection of atherosclerotic plaque

(A) Invasive catheter angiogram of coronary stenosis. (B) Curved multiplanar reconstruction prior to “Plaque Map” analysis. (C) “Plaque Map” created with fixed Attenuation ranges (necrotic core – dark green, fibrous plaque – light green and contrast orange). Due to low contrast intensity lumen is falsely identified as fibrotic plaque (white arrow). (D) Use of plaque/contrast attenuation ratios corrects for low contrast intensity.
Supplemental Figure 4. Artifacts in CT plaque classification using Plaque Maps

(A) Low attenuation of contrast in arterial lumen (L) is falsely classified as fibrous plaque by Plaque Map (left panel). VH-IVUS shows no significant plaque (plaque burden <40%) (middle panel) and histology confirms adaptive intimal thickening only with no significant atherosclerosis (right panel).

(B) Partial voluming from high attenuation contrast on the CT Plaque Map image leads to the creation of artefact (PV) fibrous plaque (light green) between lumen (orange) and necrotic core (dark green)(left panel). VH-IVUS demonstrates necrotic core (red) contact with lumen (white arrow) (middle panel) and histology confirms ruptured thin fibrous cap (white arrow) (right panel).
Supplemental references


