How to assess non-calcified plaque in CT angiography: delineation methods affect diagnostic accuracy of low-attenuation plaque by CT for lipid-core plaque in histology

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Aims
To compare the accuracy of two plaque delineation methods for coronary computed tomographic angiography (CTA) to identify lipid-core plaque (LCP) using histology as the reference standard.

Methods and results
Five ex vivo hearts were analysed by CTA and histology. LCP was defined by histology as fibroatheroma with core diameter/circumference >200 μm/≥60° and cap thickness <450 μm. In CTA, plaque was manually delineated either as the difference between the inner and outer vessel walls (Method A) or as a direct tracing of plaque (Method B). Low-attenuation plaque was defined as an area with <90 Hounsfield units. Of 446 co-registered cross-sections, 55 (12%) contained LCP. In CTA, low-attenuation plaque area was larger as assessed with Method A compared with Method B (difference: 120 ± 60%). Although low-attenuation plaque was associated with the presence of LCP, the delineation Method B yielded higher diagnostic accuracy than Method A [area under the curve (AUC): 0.831 vs. 0.780, respectively, P = 0.005]. After excluding 'normal' cross-sections by CTA (n = 117), AUC for detecting LCP became similar between both methods (0.767 vs. 0.729, P = 0.07, respectively).

Conclusion
Low-attenuation plaque in CTA is a diagnostic tool for LCP but prone to error if plaque is defined as the area between the inner and outer vessel walls and normal cross-sections are included in the assessment.

Keywords
Coronary computed tomographic angiography • Low-attenuation plaque • (Semi)-Automated plaque assessment • Plaque delineation techniques

Introduction
To date, computed tomographic angiography (CTA) remains the only modality that allows the non-invasive visualization of coronary artery disease (CAD). Exclusion of any CAD by CTA is a safe prognostic marker, whereas the presence of coronary stenosis is associated with an increased risk for coronary events.1,2 Beyond this, the morphological description of CAD by CTA has so far been of minor clinical value.

Coronary plaques with a large necrotic/lipid core and/or a thin fibrous cap are prone to rupture, leading to acute coronary events.3,4 In CTA, lipid content correlates with lower CT attenuation values when compared with fibrotic tissue.5 Accordingly, the concept of low-attenuation plaque in CTA was introduced

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and it has been shown that lipid-rich plaque has, on average, lower CT numbers as fibrotic plaque. Different CT attenuation thresholds for the definition of low-attenuation plaque were widely studied without a final conclusion regarding the optimal cut-off value. Furthermore, the impact of the chosen plaque delineation method has not yet been investigated.

Plaques can be either delineated directly or defined as the area between the inner and outer vessel walls, similar to intravascular ultrasound (IVUS) (Figure 1). The latter method is also used in all (semi)-automated plaque assessment tools for CTA and validated against IVUS for plaque burden quantification. Although (semi)-automated tools have the potential to reduce assessment time and increase inter- and intra-reader reproducibility, it remains unclear whether this delineation method affects the diagnostic accuracy of CTA to detect lipid-rich plaques.

Thus, we sought to compare the two above-described plaque delineation methods in CTA regarding the association of low-attenuation plaque in CTA to lipid-core plaque (LCP), using histology as the reference standard. Furthermore, we validated the two delineation methods for plaque burden quantification by comparing with IVUS as the reference standard.

Methods

Five isolated donor hearts with proven premature CAD were investigated. All hearts were first scanned by CT and finally processed by histology; three hearts were, in addition, imaged by IVUS. All procedures were approved by the institutional review board.

Image acquisition: CTA

Using a standardized protocol, the coronary arteries were filled with a methylcellulose-based contrast medium with 3% of a non-ionic contrast agent (Isovue 370, Bracco Diagnostics, Milan, Italy). CT images were acquired with a 64-detector row CT scanner (High-Definition, GE Discovery, CT-750HD) as described elsewhere.

Image acquisition: IVUS

Immediately after the CT scan, coronary arteries were imaged using IVUS in a pressure-perfusion system. A 40 MHz IVUS catheter (Galaxy, Boston Scientific, Boston, MA, USA) was used with a motorized automatic pullback. In post-processing, IVUS images with an axial spacing of 1.0 mm were obtained.

Image acquisition: histology

Subsequently, coronary arteries were fixated with 10% buffered neutral formalin solution. Histological analysis was performed by experts specialized in cardiovascular pathology (CVPath Laboratory, MD, USA). Briefly, 6 μm paraffin sections were obtained in millimetre increments. These were stained using Movat’s pentachrome.

Co-registration of images between modalities

Images from all three modalities were aligned by a blinded investigator using a combined approach of mathematical and anatomical co-registration. First, the distance of each image from the reference point (distal edge of the cannulas) was determined. Secondly, anatomical markers visible on all three modalities such as side branches, bifurcations, and so on were identified and applied to re-calculate image position. To each histological cross-section, one CT cross-section

Figure 1  Illustration of the two plaque delineation methods in CTA. Two different methods (Method A and Method B) were applied to delineate plaque in CTA, on which low-attenuation plaque was quantified. In Method A, plaque is defined as any pixel between the outer (blue line) and inner (yellow line) vessel walls, like most (semi)-automatic plaque assessment tools do. In Method B, the region of interest is directly traced (red line) around any clearly distinguishable non-calcified plaque portion.
and one IVUS cross-section (if available) were co-registered with 1-mm increments.

Image analysis: CTA
Using a Matlab-based in-house-developed software, two different delineation methods for quantitative assessment were applied manually to all CT cross-sections by the same investigator (6 years of experience). In Method A, the outer and inner vessel boundaries were traced and anything in-between was defined as atherosclerotic plaque. If no clearly identifiable plaque was present (‘normal’ CT cross-sections), the reader was encouraged to follow the inner and outer vessel-wall boundaries to minimize the area in-between. Method A represents the typical algorithm of a (semi)-automatic plaque quantification software. In Method B, only cross-sections with a visually identifiable non-calculated plaque portion were assessed by tracing this area directly. If the cross-sections contained no non-calculated plaque, a value of zero was given automatically. Details of the two different assessment methods are illustrated in Figure 1.

Within the region of interest, absolute amounts of pixels with attenuation < 30, < 60, and < 90 Hounsfield units (HU) were reported separately and transformed into square millimetre (mm²) for low-attenuation plaque, using the image-specific resolution.

Image analysis: IVUS
Using a planimetry software (EchoPlaque, INDEC Systems, Mountain View, CA, USA), the external elastic membrane (EEM) and vessel lumen were delineated and the area in-between was determined as the total plaque area (mm²) according to current guidelines and described in detail elsewhere.15

Image analysis: histology
Histological images were qualitatively assessed regarding the following morphological components: presence of necrotic and/or lipid core, haemorrhage, neovascularization, macrophage infiltration, and calcification (micro/spotty/sheet). Further, a quantitative assessment was performed using a dedicated software (Image) 1.44o, National Institutes of Health, Bethesda, MD, USA). If a necrotic and/or lipid core was present, the entire core area, the core circumference, and the core span were measured. Similarly, the plaque area was measured in histology, which was defined as the space between the internal elastic lamina and coronary lumen. The vessel-wall area was defined as the space between the external and the internal elastic lamina.

Study endpoints
The primary endpoint was the presence of LCP as determined in histology. Lipid-core plaque was defined as fibroatheroma, according to the modified American Heart Association (AHA) classification,18–20 with lipid core > 60% in circumferential extent, a core width of > 200 μm, and a cap which is < 450 μm in its thickness.23 The secondary endpoint was the total plaque area as derived from IVUS. In IVUS, the total plaque area is defined as the space between the EEM and vessel lumen.23

Statistical analysis
Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as percentages (frequency) if not otherwise specified. Relative difference was defined as the difference between the delineation methods defined by the mean value of Methods A and B. A mixed effects model with a random intercept accounting for repeated measures was used to assess whether the relative difference varies between applied CT attenuation thresholds.

Further, the model was extended stepwise by including (i) the co-variates ‘absence of any plaque in CTA’ and ‘presence of any calcification in CTA’ and (ii) co-variates of plaque morphology in histology.

The association of low-attenuation plaque by CTA with LCP by histology was determined in separated logistic regression models, and area under the curve (AUC) was determined as a measure for discriminative ability and compared between Methods A and B, using a non-parametric approach.22 To determine the correlation of the total plaque area between IVUS and CTA, as derived from Methods A and B, Pearson correlation coefficients (r) were used and compared using Fisher’s Z-transformation.

All statistical tests were performed by using the software SAS (version 9.2, SAS Institute, Inc., Cary, NC, USA). A two-sided P-value of < 0.05 was considered statistically significant.

Results
A total of 446 cross-sections were co-registered between CTA and histology. According to the modified AHA classification,18–20 fibroatheroma was present in 23% (n = 103). Of those, 55 cross-sections (12%) were adjudicated as LCP based on their necrotic core and fibrous cap dimensions. In CTA, 26% (n = 117) of all cross-sections were qualitatively evaluated as ‘normal’, i.e. no plaque was identified by CTA, although none of the cross-sections were evaluated as ‘normal’ in histology. In all other cross-sections (n = 329), some type of coronary atherosclerotic plaque was identified. 98% (n = 321) contained a non-calculated plaque portion, and 40% (n = 133) had any plaque calcification.

Differences in quantifying low-attenuation plaque
Throughout all thresholds, Method A led to a larger low-attenuation plaque area when compared with Method B (all P < 0.0001, Table 1), although there was a stepwise trend for lower relative differences between the two delineation methods by using higher CT attenuation cut-off values (Figure 2).

Independent of the applied CT attenuation threshold, two more factors in CTA contributed significantly to a larger relative difference between the two delineation methods: ‘normal’ cross-sections and the presence of any calcification (both P ≤ 0.01). If no plaque was present in CTA (‘normal’ cross-sections), the relative difference was 80.3% (95% CI: 69.2–91.4) larger when compared with cross-sections containing any plaque in CTA. Comparing cross-sections with and without calcification in CTA, the

Table I Low-attenuation plaque as assessed by different delineation methods (area in square millimetres)

<table>
<thead>
<tr>
<th></th>
<th>Method A</th>
<th>Method B</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area &lt; 30 HU</td>
<td>0.90 ± 1.03</td>
<td>0.14 ± 0.31</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Area &lt; 60 HU</td>
<td>2.37 ± 1.94</td>
<td>0.69 ± 0.95</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Area &lt; 90 HU</td>
<td>4.07 ± 2.89</td>
<td>1.35 ± 1.54</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Three different Hounsfield unit thresholds were applied to define low-attenuation plaque. Differences in the delineation methods are illustrated in Figure 1.
relative difference was 13% (95% CI: 3.2–24.6) larger in cross-sections containing any calcification within the plaque.

Further, with the presence of macrophages, the relative difference in low-attenuation plaque between the two delineation methods decreased [$\beta$: $-16\%$ (95% CI: $-29$ to $-3$), $P = 0.02$] and increased with increasing vessel-wall size [$\beta$: $10\%$ (95% CI: 0.4–20) per 2 SD, $P = 0.04$] (Figure 3). All other assessed plaque features in histology did not influence independently the relative difference between Methods A and B (all $P \geq 0.14$; Figure 3).

Influence of the delineation method on the association of low-attenuation plaque by CTA to LCP in histology

Low-attenuation plaque was significantly associated with LCP as determined by histology, throughout all used delineation methods (A vs. B), and applied Hounsfield unit threshold (<30, <60, and <90 HU) (Table 2). However, the discriminative ability of CTA for LCP, as expressed in AUC, was significantly higher using Method B when compared with Method A. The difference in AUC was 0.095 ($P = 0.01$), 0.055 ($P = 0.02$), and 0.051 ($P = 0.005$) for the Hounsfield unit thresholds <30, <60, and <90 HU, respectively.

After excluding ‘normal’ CTA cross-sections ($n = 117$, none of them contained LCP in the corresponding histology slides), the AUC became more similar between Methods A and B (Table 2). None of the differences in AUC yielded statistical significance anymore; however, Method B demonstrated slightly higher AUC when compared with Method A for all Hounsfield unit thresholds, which was close to statistical significance for the threshold <90 HU (difference in AUC: 0.038, $P = 0.07$).

Differences between the delineation methods for total plaque area when compared with IVUS

The average plaque area in IVUS was $7.39 \pm 3.88$ mm$^2$ in a subset of 296 cross-sections. The likelihood of identifying any plaque in CTA was strongly related to the plaque area in IVUS ($P < 0.0001$) (see Supplementary data online, Appendix S1). Because the total area in Method B captured only the non-calcified plaque portion, further analysis was restricted to cross-sections with exclusively...
non-calcified plaques \((n = 130)\). The mean total plaque area was \(10.17 \pm 3.27 \text{ mm}^2\) in IVUS, whereas it was \(8.12 \pm 2.86\) and \(3.51 \pm 1.49 \text{ mm}^2\) in CTA as derived from Methods A and B, respectively. Accordingly, the plaque area from Method A correlated significantly better with the IVUS plaque area as the plaque area from Method B \((r = 0.79\) vs. \(r = 0.61\), respectively; \(P = 0.004\) for the comparison of the correlations) (Figure 4).

**Discussion**

Both investigated delineation methods for coronary plaque in CTA yielded low-attenuation plaque areas which were significantly associated with lipid-rich plaque in histology. Tracing the coronary plaque directly in CTA led to a higher diagnostic accuracy of low-attenuation plaque as defining it by the inner and outer vessel walls, as currently used in (semi)-automated coronary plaque assessment tools. However, the total plaque area in CTA derived from the latter delineation method correlated closer with the plaque area in IVUS.

Based on the updated appropriate use criteria for CTA together with the results from the several effectiveness trials including CTA, this imaging modality will become more popular and widely used. Currently, clinical utility is limited to qualitative assessment of CAD—an important diagnostic and prognostic marker.\(^1,2\) However, by applying quantitative assessments, CTA contains more information which may potentially advance patient management and outcome.\(^23–26\)

(Semi)-automatic CTA assessment tools have been developed and validated for plaque quantification,\(^14,15,27–31\) showing a good correlation in the total plaque area between CTA and IVUS. In addition, (semi)-automatic algorithms improve the intra- and inter-reader reproducibility for the quantitative assessment.\(^16\) Also, our results indicate that the delineation method, where plaque is defined as the area between the inner and outer vessel walls (Method A), yields good estimates of the total plaque area as validated against IVUS. This method yields significantly better estimates of the total plaque area when compared with Method B, where plaque was directly traced.

However, these (semi)-automated plaque assessment tools allow not only plaque quantification, but also plaque characterization. Plaque characterization may provide important prognostic information given the fact that coronary plaques with a large lipid/necrotic core and a thin fibrous cap are at highest risk for developing an event.\(^3,4,25\) Lipid appears in CTA with a lower attenuation than fibrotic tissue. Accordingly, low-attenuation plaque has been widely investigated as a marker for plaque vulnerability.\(^6–11\)

We confirmed that low-attenuation plaque in CTA can be used as a marker for plaque with a large lipid/necrotic core, such as LCP.
This was independent of the Hounsfield unit cut-off and the applied delineation method. However, tracing the plaque directly (Method B) led to better diagnostic accuracy. Similarly, in a recent study comparing CTA against IVUS, manual delineation of non-calcified plaque (similar to our Method B) led to a high AUC as of 0.9. Improved diagnostic accuracy would improve patient management and enable to monitor the change in plaque characterization as a result of drug treatment.

In contrast, most studies follow the approach of Method A, where plaque is defined as the area between the inner and outer vessel walls. Today, this delineation method is used in all commercially available (semi-)automated plaque assessment tools. However, we demonstrated that this method was inferior regarding the diagnostic accuracy. We further demonstrated that the reasons for the inferiority are mostly driven by the following four factors: (i) including cross-sections without any plaque (‘normal’) by CTA; (ii) the presence of calcification; and (iii) increasing vessel-wall size; although the vessel wall consists of fibrotic tissue, it also includes pixels with low CT attenuation; because the outer vessel-wall boundary is difficult to differentiate in CTA and it cannot be excluded that the adventitia and the adjacent pericardial fat (attenuation below ~ 45 HU) were included within the region of interest; and (iv) the presence of macrophages, which is an interesting finding because they cannot be visualized by CTA.

By restricting the analysis to cross-section containing any plaque by CTA, the difference in diagnostic accuracy between the two delineation methods became non-significant. However, excluding ‘normal’ cross-sections by CTA from the analysis may improve specificity but may alter sensitivity. Moreover, plaque is only visible on CTA if it reaches a particular size.

**Clinical implications**

The utilization of (semi-)automated assessment tools in CTA has the potential to advance patient management and outcome. However, plaque delineation methods must be carefully evaluated before routine clinical implementation. Applying Method A throughout the entire coronary tree, regardless of whether plaque is visible or not, may provide reliable results for plaque quantification. However, for plaque characterization, plaque should be either directly traced (Method B) or segments without visible plaque should be excluded from analysis when using the inner and outer vessel-wall boundaries for delineation (Method A). The accurate detection and delineation of low-attenuation plaque may improve our ability to diagnose patients with acute coronary syndrome presentations, to predict future major adverse cardiovascular events, and to monitor therapeutic interventions.

**Limitations**

Our results must be considered under several limitations. This study was performed in an ex vivo setting with a limited sample size and not including cardiac motion. Although there is no evidence that the difference between the delineation methods varies with cardiac motion or different CT vendors, this fact must be considered for the translation into in vivo. Further, the boundaries were drawn manually and may be objective for human error. However, in (semi-)automated plaque delineation, still a large portion of the automatically fitted boundaries must be corrected manually. Future ex vivo and in vivo research is warranted to assess the generalizability of our findings.

**Conclusion**

Low-attenuation plaque by CTA permits detection of LCP as defined by histology. However, it is prone to error if plaque is defined as the area between the inner and outer vessel walls, and normal cross-sections are included in the assessment. Future (semi-)automated plaque assessment tools should eliminate cross-sections without visually apparent plaque from measurements of low-attenuation plaque.

**Supplementary material**

Supplementary data are available at European Heart Journal – Cardiovascular Imaging online.

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**References**


Delineation methods affect diagnostic accuracy of CTA


