In Vivo Characterization and Quantification of Atherosclerotic Carotid Plaque Components With Multidetector Computed Tomography and Histopathological Correlation

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Objective—In a previous in vitro study we have demonstrated that atherosclerotic plaque components can be characterized with multidetector computed tomography (MDCT) based on differences in Hounsfield values (HV). Now we evaluated the use of MDCT in vivo to characterize and quantify atherosclerotic carotid plaque components compared with histology as reference standard.

Methods and Results—Fifteen symptomatic patients with carotid stenosis (>70%) underwent MDCT angiography before carotid endarterectomy (CEA). From each CEA specimen 3 histological sections and corresponding MDCT images were selected. The HV of the major plaque components were assessed. The measured HV were: 657 ± 416 HU, 88 ± 18 HU, and 25 ± 19 HU for calcifications, fibrous tissue, and lipid core, respectively. The cut-off value to differentiate lipid core from fibrous tissue and fibrous tissue from calcifications was based on these measurements and set at 60 HU and 130 HU, respectively. Regression plots showed good correlations (R² > 0.73) between MDCT and histology except for lipid core areas, which had a good correlation (R² = 0.77) only in mildly calcified (0% to 10%) plaques.

Conclusions—MDCT is able to quantify total plaque area, calcifications, and fibrous tissue in atherosclerotic carotid plaques in good correlation with histology. Lipid core can only be adequately quantified in mildly calcified plaques.

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Key Words: carotid stenosis □ computerized tomography □ magnetic resonance imaging □ imaging

The severity of luminal stenosis, caused by the atherosclerotic plaque in the carotid bifurcation, is an important risk factor for (recurrent) stroke and is used in therapeutic decision making: ie, patients with symptomatic or asymptomatic carotid stenosis above a certain degree are considered candidates for carotid intervention such as carotid endarterectomy (CEA) or stent placement.1 However, morphology studies on carotid atherosclerotic plaque have revealed that plaque morphology could be an important additional feature in the risk assessment of patients with carotid stenosis.2,3

Computed tomography angiography (CTA) is an accurate modality to grade the severity of stenosis4 and is increasingly used in the evaluation of stroke patients. The question then arises whether CT can also provide detailed information about plaque morphology.

Earlier studies in which 3-mm-thick single-slice CT images were compared with histology sections of CEA specimens yielded confusing results.5,6 Multidetector CT (MDCT) allows evaluating carotid atherosclerosis with thinner slices (0.5 to 1.0 mm) and less volume averaging. More detailed analysis of plaque composition may now become possible.

A previous in vitro validation study showed that thin-section MDCT is capable of characterizing and quantifying calcifications and lipid core regions in CEA specimens based on differences in Hounsfield values (HV).7 However, in vitro studies have inherent limitations attributable to the presence of air around the specimen and the absence of contrast in the vessel lumen. The purpose of this study was to assess the ability of in vivo thin-section MDCT to characterize and quantify carotid plaque compared with histology as reference standard.

Materials and Methods

Subjects

Fifteen patients (6 male, 9 female; mean age 70.3 years, range 62 to 84 years) with a transient ischemic attack (TIA) or minor stroke and with severe ipsilateral carotid atherosclerotic disease (>70%) on CTA underwent CEA within three months (mean: 2.2 ± 0.9 months) after CTA. All but one patient (who experienced a TIA) were asymptomatic between CTA and CEA. The CEA specimens were...
collected and stored in 4% formaldehyde. The Institutional Review Board approved the study, and patients gave written informed consent.

**Scanning and Image Reconstruction**

Scanning was performed on a 16-slice MDCT scanner (Siemens, Sensation 16) with a standardized optimized contrast-enhanced protocol (120 kVp, 180 mAs, collimation 16×0.75 mm, table feed 12 mm/rotation, pitch 1). Image reconstructions were made with field of view 100 mm, matrix size 512×512 (real in-plane resolution 0.6×0.6 mm), slice thickness 1.0 mm, increment 0.6 mm, and with an intermediate reconstruction algorithm.7

**Histology Preparation**

The 15 CEA specimens were partly decalcified and embedded in paraffin. Three histological sections were obtained per 1-mm and stained with hematoxylin-eosin, Sirius Red, and elastic-Van Gieson, respectively.

**MDCT and Histology Matching**

The shape of the lumen and vessel wall, the location of the bifurcation, and the presence of calcifications in the plaque were used for matching. From each specimen, 3 histological sections and corresponding MDCT images were selected for analysis: the most stenotic site and one site proximally and one site distally within 5 to 10 mm. A minimum distance of 5 mm between histological sections was chosen to obtain a heterogeneous data set and to minimize selection bias.9 Because not every histological section was of good quality, some sections had to be chosen at a larger distance than the preferred 5 mm.

**Differentiation of Plaque Components**

Within the selected histological sections, regions with one predominant plaque component (calcifications, fibrous tissue, or lipid core) were determined. The HV of these regions were measured by drawing a region of interest (ROI) in the center of the same region in the corresponding MDCT images. Care was taken to place the ROI in lipid core and fibrous tissue regions at a distinct distance from calcified tissue and vessel lumen, to avoid partial volume effects. Based on these measurements a cut-off value was determined to differentiate lipid core from fibrous tissue.

**Size of Different Plaque Components**

The size of the 3 predominant plaque components was assessed. One observer measured the areas within the histological sections, after reviewing them with a histopathologist. One month later the same observer, who was blinded for the histology results, assessed the areas within the MDCT images. Plaque component areas were expressed as percentages of the total plaque area, because histological preparation leads to plaque shrinkage.10

The histological sections were analyzed with a microscopy-image-analysis-system (Clemex vision 3.5, Clemex Technologies, Inc). This system allows to assess (in a semiautomatic way) the total plaque area, calcified area, and fibrous tissue area (which included also the tunica media); the remaining tissue (lipid, hemorrhage, and necrotic debris) was then considered to be lipid core.7

In the corresponding MDCT images, the total plaque area and the area of each plaque component was determined, using a custom-made plug-in for the freely available software ImageJ (Rasband, National Institute of Mental Health). This plug-in assessed the total number of pixels (=plaque + lumen area) and the number of pixels of different HV ranges (=different plaque component areas) within a manually drawn ROI (Figure 1). Each HV range was considered to represent a different plaque component and was given a different color within the MDCT image. By multiplying the number of pixels of each HV range by the pixel size, total plaque area and plaque component areas were determined.

The cut-off point between lumen and atherosclerotic vessel wall was set at 200 Hounsfield Units (HU). This cut-off point was chosen to compensate for partial volume effects that appear at the border between atherosclerotic plaque and contrast-enhanced lumen, which has a mean HV at the carotid bifurcation of ≈400 HU with the described scan protocol.8 The cut-off point between calcifications and non-calcified tissue was set at 130 HU, the value currently used in calcium scoring.11 When calcified areas bordered the lumen and merged with the lumen, lumen area and calcifications were separated by manual drawing. The cut-off point between fibrous tissue and lipid was based on the HV measurements in the present study in regions that showed predominant lipid core or fibrous tissue.

To evaluate inter- and intraobserver variability in MDCT area measurements, a second observer independently performed the area measurements whereas the first observer reassessed the areas after 4 months.

**Detection of Lipid Core in MDCT Images**

To investigate the interpretation of hypodense regions within atherosclerotic plaque in MDCT images, each hypodense region was divided in 3 different regions based on a range of HV (<0 HU, 0 to 30 HU, 30 to 60 HU). For each range of HV the number of hypodense regions in the MDCT images was assessed and the results were subsequently compared with the histological section. On histology, hypodense regions were true-positive for lipid core if the whole region fell within a lipid core area (ie, lipid, hemorrhage, or necrotic debris) and false-positive for lipid core if these regions included (besides lipid core) calcified or fibrous tissue. Hypodense regions along the vessel wall border were counted separately because they were caused by inadequate positioning of the outer contour with inclusion of periarterial fat in the analysis.

**Statistics**

Data are presented as the mean±SD. Continuous data were compared with Student t test or paired Student t test. A probability value <0.05 was considered to indicate statistical significance. Relationships between area measurements in MDCT images and histology were evaluated with linear regression analysis and Bland–Altman plots. The degree of observer variability is presented both with Bland–Altman plots and a coefficient of variation.

**Results**

In 14 of 15 endarterectomy specimens it was possible to match MDCT images with corresponding histological sections (Figure 2). One specimen had to be excluded because of dental streak artifacts in the MDCT images. In another specimen, because of its limited length, only 2 matched histological sections and MDCT images could be evaluated. Hence, 41 matched levels were available for evaluation.

**Differentiation of Plaque Components**

In Table 1 the number of HV measurements in predominant calcified fibrous tissue and lipid core regions per histological section, the total number of HV measurements, and the mean HV of each plaque component are given. A significant difference in HV between fibrous tissue and lipid core was found (P<0.001). In 21 histological sections from 11 different CEA specimens, predominant regions of both fibrous tissue and lipid core were present in the same slice. In all cases the HV of lipid core was lower than the HV of fibrous tissue.

Based on the distribution of measured HV of lipid core (range, ~20 to 60 HU) and fibrous tissue (range, 60 to 140 HU) in the MDCT images, 60 HU was determined as the cut-off point to differentiate lipid core from fibrous tissue.
Size of Different Plaque Components

All 41 matched levels of histological sections and MDCT images were used for area measurements (Table 2). Total plaque area and calcified area in MDCT images were significantly larger than the histological total plaque area and calcified area (P < 0.001), whereas fibrous area in MDCT images was significantly smaller than the histological fibrous area. There was no significant difference between MDCT and histology for lipid core area measurements.

The correlation between histology and MDCT for total plaque area, calcified area, and fibrous tissue area was good (R² = 0.73, 0.74, 0.76, respectively; P < 0.001), whereas fibrous area in MDCT images was significantly smaller than the histological fibrous area. There was no significant difference between MDCT and histology for lipid core area measurements.

The correlation between histology and MDCT for total plaque area, calcified area, and fibrous tissue area was good (R² = 0.73, 0.74, 0.76, respectively; P < 0.001), whereas the correlation for lipid core area was poor (R² = 0.24; P < 0.002; Figure 3a). However, when we performed an exploratory analysis by evaluating the lipid core area data in severely and mildly calcified plaques and changed the cut-off value between severely and mildly calcified plaques (<30%, <20%, <10%, or 0% calcified, respectively) the correlation between lipid core areas on histological sections and in MDCT images improved for the mildly calcified plaques (R² = 0.50, 0.62, 0.77, and 0.81, respectively; P < 0.001), whereas it remained poor for the severely calcified plaques (Figure 3b).

The difference between MDCT measurements and histological measurements of total plaque area and calcified area became larger as the total plaque area and the calcified area increased, respectively. The latter is explained by the fact that the blooming effect of calcifications leads to overestimation of calcifications. In addition, this effect increases with the size of the calcification. However, the measurement of the size of the total plaque area is less affected by this blooming effect. The difference between fibrous tissue and lipid core areas assessed on histology and in MDCT images could not be related to their size (please see the supplementary materials, available online at http://atvb.ahajournals.org).

Inter- and Intraobserver Variability

The MDCT area measurements of the two observers showed significant differences (P < 0.05) for the assessment of total plaque area, fibrous tissue area, and lipid core area. The lumen area and calcified area were assessed with no significant difference (P > 0.05). The intraobserver measurements showed significant differences (P < 0.05) for the assessment of total plaque area. The lumen area, calcified area, fibrous tissue area, and lipid core area were assessed with no significant difference (P > 0.05).

The Bland–Altman plots of the area measurements of the two observers showed that observer variability is mainly caused by variability in the manual outlining of the outer vessel wall.
The inter-observer coefficients of variation for the absolute measurement of lumen, plaque, calcified, fibrous tissue, and lipid core areas were: 4%, 19%, 16%, 21%, and 40%, respectively, and the interobserver coefficients of variation for the relative measurement (%) of calcified fibrous tissue and lipid core areas were: 26%, 10%, and 20%, respectively. The intraobserver coefficients of variation for the absolute measurement of lumen, plaque, calcified, fibrous tissue, and lipid core areas were: 3%, 8%, 8%, 11%, and 15%, respectively, and the intraobserver coefficients of variation for the relative measurement (%) of calcified fibrous tissue and lipid core areas were: 26%, 10%, and 20%, respectively. The intraobserver coefficients of variation for the absolute measurement of lumen, plaque, calcified, fibrous tissue, and lipid core areas were: 3%, 8%, 8%, 11%, and 15%, respectively, and the intraobserver coefficients of variation for the relative measurement (%) of calcified fibrous tissue and lipid core areas were: 26%, 10%, and 20%, respectively.

Table 1. Total number of HV Measurements and Mean HV (mean±SD) for Predominant Calcified, Fibrous Tissue and Lipid Core Regions per Histological Section

<table>
<thead>
<tr>
<th>No. of Measurements Per Section</th>
<th>Calcified</th>
<th>Fibrous</th>
<th>Lipid core</th>
</tr>
</thead>
<tbody>
<tr>
<td>0×</td>
<td>15</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>1×</td>
<td>20</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>2×</td>
<td>6</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>3×</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Total No. of measurements</td>
<td>32</td>
<td>53</td>
<td>31</td>
</tr>
<tr>
<td>Mean HV (HU)</td>
<td>657±416</td>
<td>88±18</td>
<td>25±19</td>
</tr>
</tbody>
</table>

The difference in HV between fibrous tissue and lipid core is significant (P<0.001).

Table 2. Total Plaque Area and Plaque Component Areas (mean±SD) in 41 Matched MDCT Images and Histological Sections

<table>
<thead>
<tr>
<th></th>
<th>Total Plaque Area (mm²)</th>
<th>Calcified Area (%)</th>
<th>Fibrous Area (%)</th>
<th>Lipid Core Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDCT</td>
<td>50.1±22.4</td>
<td>24.2±22.9</td>
<td>52.9±19.4</td>
<td>22.9±16.0</td>
</tr>
<tr>
<td>Histology</td>
<td>34.7±12.3</td>
<td>8.1±10.3</td>
<td>66.6±17.2</td>
<td>25.8±15.3</td>
</tr>
</tbody>
</table>

All area measurements in the MDCT images, except the lipid core measurements, were significantly different (P<0.001) from the histological data.
the relative measurement (%) of calcified fibrous tissue and lipid core areas were: 14%, 7%, and 14%, respectively.

**Detection of Lipid Core in MDCT Images**

Analysis of hypodense regions showed that 54 hypodense regions were present in the HV range of <30 HU: 28 regions were true positive, 26 regions were located at the vessel wall borders. With a HV range of 0 to 30 HU, 118 hypodense regions were present: 49 regions were true positive, 3 regions false-positive, and 66 regions were located at the vessel wall borders. A HV range of 30 to 60 HU, 144 hypodense regions were present: 28 regions were true positive, 95 were false-positive, and 21 regions were located at the vessel wall borders.

**Discussion**

This study shows that the HV measured in the center of fibrous-rich regions and lipid core is significantly different. This confirms the results of our earlier in vitro study. In addition, the present study reveals how to interpret very hypodense regions (<30 HU) in the center of atherosclerotic carotid plaque in MDCT images; these regions are associated with the presence of a lipid core (ie, lipid, hemorrhage, or necrotic debris). To our knowledge, this in vivo MDCT study is the first to use differences in HV between plaque components to quantify atherosclerotic carotid plaque and its components, and to correlate the findings with histology: MDCT was capable of quantifying total plaque area, calcified area, and fibrous tissue area and lipid core area in mildly calcified plaques. The interobserver variability in area measurements was moderate (range, 4 to 40%), and the intraobserver variability was good (range, 3 to 15%).

The present study shows that the HV of calcifications, fibrous tissue, and lipid core differ significantly from each other, which is in concordance with earlier carotid and coronary CT studies. These differences allow quantification of total plaque area and plaque component areas.

Total plaque area acquired in MDCT images was 30% larger than the histological total plaque area; this is in accordance with reported plaque shrinkage of 19% to 25% attributable to histological preparation. The correlation between total plaque area measurements is strong, although observer variability can be improved when using new methods of measurement (eg, automated vessel-wall contour detection, volume measurements instead of area measurements). Therefore, MDCT-based plaque volume assessment may prove to be ready for use in follow-up studies, and the predictive value of plaque volume for stroke might be assessed.

The quantification of calcifications has been extensively reported and, although the calcified mass is reported to be overestimated with CT, accurate event prediction has been described in the coronaries: the greater the calcified mass, the higher the risk of an acute event.

However, a similar study in the carotid arteries describes the protective value of a higher degree of calcification. This suggests that the degree of calcification should be regarded as a reflection of the presence of more localizations of atherosclerosis rather than the instability of calcified plaques. In our study calcifications were also overestimated. Nevertheless, there was a strong correlation with histology. The main problem with the overestimation of calcifications (mainly attributed to the blooming effect of calcifications) is not the imprecise quantification of the calcium mass but its influence on optimal characterization of the noncalcified part of the plaque.

Quantification of fibrous tissue has not been done previously with MDCT. The present study shows, however, that this is possible with a strong correlation to histology. Because of the fact that fibrous tissue is a stabilizing feature of an atherosclerotic plaque, the quantification could qualify fibrous tissue as an important predictor of plaque stability.

Accurate assessment of the presence of lipid core is of interest for risk prediction because lipid core is one of the conclusive features of plaque instability.
an unstable plaque. The present study shows that very hypodense regions are associated with the presence of a lipid core. Nevertheless, the correlation between histological sections and MDCT images for lipid core area measurements was poor, although only for heavily calcified plaques. The lack of correlation is explained in our opinion by the blooming effect of calcifications in the MDCT images. This blooming effect, as earlier explained, overshadows parts of the soft plaque and hampers the correct characterization of those parts. The exploratory analysis we performed shows indeed a better correlation for soft plaque regions (lipid core and fibrous regions) when less calcifications are present and more of the soft plaque can be correctly classified. In addition, the lack of correlation in lipid core area measurements can be explained by the relatively high observer variability for these measurements. This might be a result of possible inclusion of periarterial fat in the analysis by drawing too large outer vessel wall contours by the observers.

A recent prospective MRI study by Takaya et al. showed a positive association between a higher percentage lipid-necrotic core and a higher event rate. Whether the same association exists between with MDCT identified and/or quantified lipid core needs to be established in prospective MDCT studies. Because severe stenosis will be treated already, risk assessment in patients with moderate degree of stenosis is more important. Fortunately, these patients have less calcifications. Thus, MDCT based lipid core evaluation should not be problematic.

Limitations
The first limitation of this study concerns histology, which is considered the reference standard for the assessment of plaque components with MDCT. However, it is limited for this purpose, because of several reasons: (1) Surgical plaque extirpation is not always done in toto, therefore small parts of the vessel wall cannot be taken into account during histological analysis. (2) During slicing histological sections can become damaged, and this may result in underestimation of histological plaque burden. (3) Because to necessary routinely performed decalcification, small calcifications may be missed and larger calcifications may be underestimated by histological area measurements. However, no hyperdense regions in the MDCT images (besides the contrast filled lumen) were determined that could not be correlated to the presence of calcifications. If small calcifications have been missed on histology because of decalcification, they were also too small to be depicted in MDCT images. The strong correlation between calcified areas in MDCT images and histology and the systematic overestimation of calcium in MDCT images clarifies that the possible underestimate of larger calcifications is of little influence. (4) Because the resolution of histological sections (±7×7×5 μm) cannot be achieved with MDCT (±0.6×0.6×0.6 mm), histological morphology will show too much detail compared with MDCT-based morphology. (5) Plaque shrinkage that occurs during histological preparation alters the gross morphology of the plaque, and it is unknown to what extent each component contributes to the plaque shrinkage.

The second limitation concerns atherosclerotic plaques with severe calcifications. These calcifications will hamper correct quantification of lipid core. However, our exploratory analysis has shown that these hampering effects are less in moderately calcified plaques. Fortunately, this will be the main plaque type in the patient population that will benefit most of stroke prediction based on plaque morphology.

A third limitation concerns the identification of hemorrhage and thrombus as recommended in a recent review on the performance and reporting of studies on carotid plaque imaging versus histology. MDCT can differentiate between calcifications, fibrous tissue, and lipid core, but because hemorrhage and thrombus cannot be distinguished reliably from lipid they are not reported separately.

A fourth limitation lies in the fact that the assessment of the cut-off value between fibrous tissue and lipid core was performed in the same data set in which the quantification of components was performed. We choose this approach, because availability of specimens was low and we did not want to lower the number of specimens available for quantification. We recognize that by doing this we report the highest achievable level of correlation.

The last limitation concerns the passing of 2.2 months between CTA and CEA. During this period the atherosclerotic plaque can have changed from what was recorded in the MDCT images. However, these changes will have had a negative effect on the found correlations and the actual correlations might therefore be even better.

Conclusions
Although CTA is established as an accurate modality to grade the severity of stenosis, the results regarding the characterization of atherosclerotic disease in the carotid bifurcation are confusing. The present study shows that MDCT is capable of characterizing and quantifying plaque burden, calcifications, and fibrous tissue in atherosclerotic carotid plaque in good correlation with histology, and that lipid core can be adequately quantified in mildly calcified plaques. Furthermore the MDCT-based assessment of atherosclerotic plaque component quantities was possible with a moderate observer variability. Further studies are required to determine whether MDCT-assessed plaque parameters are important predictors of stroke or can function as secondary end points in pharmacological studies of plaque regression.

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Disclosures
None.

References


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Figure I. Bland-Altman plot of total plaque area and plaque component areas in MDCT images and histological sections. The horizontal lines express the mean difference and the mean difference ± 2 standard deviations.
Figure II. Bland-Altman plot of total plaque area and plaque component areas assessed by two observers. The horizontal lines express the mean difference and the mean difference ± 2 standard deviations.