High-Resolution CT Imaging of Carotid Artery Atherosclerotic Plaques


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BACKGROUND AND PURPOSE: Plaque morphologic features have been suggested as a complement to luminal narrowing measurements for assessing the risk of stroke associated with carotid atherosclerotic disease, giving rise to the concept of “vulnerable plaque.” The purpose of this study was to evaluate the ability of multidetector-row CT angiography (CTA) to assess the composition and characteristics of carotid artery atherosclerotic plaques with use of histologic examination as the gold standard.

MATERIALS AND METHODS: Eight patients with transient ischemic attacks who underwent carotid CTA and “en bloc” endarterectomy were enrolled in a prospective study. An ex vivo micro-CT study of each endarterectomy specimen was obtained, followed by histologic examination. A systematic comparison of CTA images with histologic sections and micro-CT images was performed to determine the CT attenuation associated with each component of the atherosclerotic plaques. A computer algorithm was subsequently developed that automatically identifies the components of the carotid atherosclerotic plaques, based on the density of each pixel. A neuroradiologist’s reading of this computer analysis was compared with the interpretation of the histologic slides by a pathologist with respect to the types and characteristics of the carotid plaques.

RESULTS: There was a 72.6% agreement between CTA and histologic examination in carotid plaque characterization. CTA showed perfect concordance for calcifications. A significant overlap between densities associated with lipid-rich necrotic core, connective tissue, and hemorrhage limited the reliability of individual pixel readings to identify these components. However, CTA showed good correlation with histologic examination for large lipid cores ($x = 0.796; P < .001$) and large hemorrhages ($x = 0.712; P = .102$). CTA performed well in detecting ulcerations ($x = 0.855$) and in measuring the fibrous cap thickness ($R^2 = 0.77; P < .001$).

CONCLUSION: The composition of carotid atherosclerotic plaques determined by CTA reflects plaque composition defined by histologic examination.

Luminal narrowing is the standard parameter used in reporting the extent and severity of carotid artery stenosis. The widespread use of this measure is based primarily on the results of several randomized clinical trials that demonstrated a reduction in the risk for ischemic stroke in patients with luminal stenosis of 70% or greater (assessed on conventional angiograms), after carotid endarterectomy compared with medical treatment alone.1-4 However, carotid stenosis of 70% or more occurs in less than 10% of patients, whereas less than 70% of carotid stenosis is extremely frequent in the general population (70% in men and 60% in women 64 years of age).3,6 In patients with less than 70% carotid stenosis, high-resolution lumenography fails to provide any insight into the associated risk for stroke, because angiography is able to detect atherosclerosis only when more than 40% of the area of the vessel wall is occupied by the plaque.7

Plaque morphologic features and composition have been suggested as a complement to luminal narrowing measurements for assessing carotid atherosclerotic disease, giving rise to the concept of “vulnerable plaque.” Several morphologic features have been reported as being associated with an increased risk for stroke, the most studied descriptor being the common carotid artery (CCA) intima-media thickness.5,6,13-17 Carotid plaques with a thin fibrous cap and a large lipid core are also considered to increase the risk for stroke,18-20 as are ulcerated plaques.20 In contrast, plaques with high calcium content, especially when located superficially, are thought to be associated with a lower risk for stroke.21

Noninvasive in vivo imaging of carotid atherosclerotic plaques holds considerable promise for clinical decision making and treatment.18,22,23 Such imaging has classically been achieved with sonography5,6,13-17 and MR imaging.5,24-29 It is surprising that only a few studies have evaluated carotid wall descriptors with CT,30-32 though CT angiography (CTA) is a well-established technique frequently used to assess carotid stenosis.30,33,34 Previous studies that explored CTA as a means of imaging atherosclerotic plaques have involved older-generation, single-section CT scanners31,32,35-40 and have usually focused on 1 single component, such as calcium.36,38,39,41

The goal of this study was to evaluate the ability of modern, multidetector-row, isotropic resolution CTA studies to assess
the histologic composition (including noncalcified components) and characteristics of carotid artery atherosclerotic plaques with use of histologic examination as the gold standard.

Methods

Study Design
Eight patients with transient ischemic attacks (TIA) underwent a CTA study, were found to have more than 50% carotid stenosis, and were scheduled for carotid endarterectomy as part of their standard of care. They were enrolled in a prospective study approved by our institutional review board. As part of the research protocol, patients were asked to provide permission for their preoperative CTA study and endarterectomy specimen to be used for research purposes. The endarterectomy specimens were excised en bloc according to a technique described previously in the literature. An ex vivo micro-CT study of each specimen was obtained, followed by ex vivo histologic examination. Two analyses, 1 quantitative and the other qualitative, were performed, comparing in vivo CTA to histologic examination, the gold standard for noncalcified carotid wall components, and with ex vivo micro-CT, the reference for carotid wall calcium (specimens were decalcified before histologic sectioning). Details of each analysis are described below and are derived from the methodology recommended by Lovett et al for comparing carotid plaque imaging to histologic features.

In Vivo CTA Imaging Protocol
The CTA studies were obtained on a 16-section CT scanner (GE Healthcare, Milwaukie, Wis). The image acquisition protocol was as follows: spiral mode, 0.6-second gantry rotation; collimation, 16 × 0.625 mm; pitch, 1.375:1; section thickness, 0.625 mm; reconstruction interval, 0.5 mm; and acquisition parameters: 120 kVp/240 mA. A caudocranial scanning direction was selected, covering from the midchest to the vertex. Iohexol (Omnipaque; Amersham Health, Princeton, NJ; 300 mg/mL of iodine) 70 mL was injected to an antecubital vein with a power injector at a rate of 4 mL/s. Optimal timing of the CTA acquisition was achieved with use of a test bolus technique.

Ex Vivo Micro-CT Imaging Protocol of the Carotid Endarterectomy Specimens
The carotid endarterectomy specimens were imaged with a VivaCT 40 micro-CT scanner (Scanco Medical, Southeastern, Pa) with the following parameters: 70 kVp, 160 μA, 30-μm isotropic resolution.
method described previously. Grids of 2 × 2-mm squares were electronically drawn on the reformatted images in the same orientation and location as on the corresponding histologic slides. To avoid any bias, the process of orienting and localizing CTA and micro-CT images with histologic examination was performed by a separate reviewer, before and independent of the qualitative and quantitative analyses described below.

Quantitative Analysis
A pathologist, who was blinded to the CTA images, independently reviewed the histologic slides in combination with the matching micro-CT images and evaluated the composition of the carotid walls in each 2 × 2-mm square, as delineated by the slide grids. The pathologist’s assessment was based on examination of the slides under a microscope. The histologic slides were used as the gold standard for identifying regions of connective tissue, lipid-rich necrotic core, and hemorrhage. Because specimens were decalcified during fixation before histologic sectioning, the micro-CT images served as the gold standard for identifying calcified regions. The pathologist outlined and labeled the regions corresponding to these 4 components (connective tissue, lipid-rich necrotic core, hemorrhage, and calcifications) on the corresponding digitized images. The tissue classes were defined before classification as follows: regions of collagen strands and elastic fibers, connective tissue matrix, and proteoglycans were termed connective tissue; regions containing cellular debris, a disorganized mass of lipid material and/or cholesterol crystals, cholesterol clefts, and lipid-laden foam macrophages were called lipid-rich necrotic core; and regions with blood products or calcifications were called hemorrhage and calcifications, respectively. The areas of each of these regions were computed, and the percentages of connective tissue, lipid-rich necrotic core, hemorrhage, and calcifications in each of the 2 × 2-mm squares were calculated.

The in vivo CTA reformatted images were reviewed independent of the histologic slides. The average CT Hounsfield attenuation was recorded in each of the 2 × 2-mm squares electronically drawn on the reformatted images in the same orientation and location as on the corresponding histologic slides. Using a linear mixed model of the average CT Hounsfield attenuation in each of the 2 × 2-mm squares (outcome) with respect to the percentages of connective tissue, lipid-rich necrotic core, hemorrhage, and calcifications in the corresponding histologic squares (predictors), with a random effect for patient, and assuming the errors were normal, we determined the mean Hounsfield attenuation for each histologic component (connective tissue, lipid-rich necrotic core, hemorrhage, and calcifications), as well as the 95% confidence intervals for these densities. This linear mixed model with a random effect for patient built interpatient correlation into the calculation of these densities and an assessment of their variability. Because the variances for the different components were similar, the optimal cutoff for differentiating between histologic components was determined to be the halfway Hounsfield attenuation between the mean densities for each of the components.

Qualitative Analysis
We developed an automated classification computer algorithm that segments the inner (luminal) and outer contours of the carotid artery walls from the in vivo CTA datasets; then, with the Hounsfield attenuation thresholds calculated in the quantitative analysis as described above, the “type” of each image pixel (connective tissue, lipid-rich necrotic core, hemorrhage, and calcifications) located within the carotid wall is assigned. Each of the CTA reformatted images was analyzed by this algorithm, and a color overlay was created affording a visual display of the composition of the carotid wall for each CTA image (Figs 1–4).

A neuroradiologist reviewed these color overlays on the CTA reformatted images (as displayed in Figs 1–4) and characterized the type of atherosclerotic plaque and the stage of lesion development in each quadrant of the carotid wall (0–3 hours, 3–6 hours, 6–9 hours, and 9–12 hours) using a system derived from the American Heart Association (AHA) classification system and adapted for noninvasive image data, such as CT images (Table 1). This adaptation was accomplished by combining type I and II lesions, as well as type IV and Va lesions, as proposed previously. Ulceration was defined by the presence of large obvious excavation (≥2 mm in depth) on the surface of the plaque, with a well-defined back wall at its base. The maximal thickness of the plaque and the minimal thickness of the
fibrous cap, as automatically calculated by the computer algorithm for each quadrant of each CTA image, were recorded for the CTA images that had a corresponding histologic section. Of note, the fibrous cap thickness measured by the software is the radial distance to the most superficial core of nonconnective tissue (lipid, blood, or calcium). If no lipid core, hemorrhage, or calcium is present in the intraplaque hemorrhage. Cutoff densities between lipid-rich necrotic core and connective tissue, connective tissue and hemorrhage, and 177.1 HU between lipid-rich necrotic core and connective tissue. There was significant overlap in CT Hounsfield densities for each of the 2 × 2-mm squares that were electronically drawn on the CT reformatted images and considered in the linear regression model with respect to the percentages of connective tissue, lipid-rich necrotic core, hemorrhage, and calcifications in the corresponding histologic and micro-CT squares. The results of the linear mixed model (ie, mean Hounsfield attenuation for each histologic component and the 95% confidence intervals for these densities) are displayed in Table 2.

There was significant overlap in CT Hounsfield densities between lipid-rich necrotic core and connective tissue. There was also some overlap between connective tissue and hemorrhage. Cutoff densities between lipid-rich necrotic core and connective tissue, connective tissue and hemorrhage, and hemorrhage and calcifications were determined as the halfway Hounsfield attenuation between the average densities for each of the components: 39.5 Hounsfield units (HU) between lipid-rich necrotic core and connective tissue, 72.0 HU between lipid-rich necrotic core and connective tissue, and 217.5 HU between lipid-rich necrotic core and connective tissue.

### Quantitative Analysis

The mean CT Hounsfield attenuation was measured for each of the 2 × 2-mm squares that were electronically drawn on the CT reformatted images and considered in the linear regression model with respect to the percentages of connective tissue, lipid-rich necrotic core, hemorrhage, and calcifications in the corresponding histologic and micro-CT squares. The results of the linear mixed model (ie, mean Hounsfield attenuation for each histologic component and the 95% confidence intervals for these densities) are displayed in Table 2.

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### Qualitative Analysis

The comparison of CT classification and gold standard histologic classification of type of atherosclerotic plaque and stage of lesion development according to the system derived from the AHA classification system43.44 is displayed in Table 3. There was an overall 72.6% agreement between CTA and histologic examination, corresponding to an unweighted $\kappa$ of 0.67.6. ($P < .001$) (Figs 1 and 2). For large calcifications (Vb plaques), CTA classified the lesion in perfect concordance with histologic features (Table 4).
Patients with occluded vessels do not typically proceed to endarterectomy, and there was no thrombosed plaque (Vlc) among the 8 subjects in our study group.

The mean of the minimal fibrous cap thickness was 0.9 ± 1.1 mm (range, 0.0–5.6 mm) on histologic examination, and 1.1 ± 1.0 mm (range, 0.0–6.6 mm) on CTA. Linear regression between CTA and histologic examination in fibrous cap thickness was excellent (P < .001), with a slope of 0.86 (95% confidence interval, 0.77–0.96), an intercept of 0.3 mm (95% confidence interval, 0.2–0.5) and a coefficient of correlation $R^2 = 0.77$.

The mean of the maximal carotid wall thickness was 3.4 ± 2.4 mm (range, 0.1–10.5 mm) on histologic examination, and 5.1 ± 2.8 mm (range, 0.4–11.6 mm) on CTA. Linear regression between CTA and histologic examination in carotid wall thickness was significant (P < .001), with a slope of 0.98 (95% confidence interval, 0.86–1.11), an intercept of 1.8 mm (95% confidence interval, 1.3–2.3) and a coefficient of correlation $R^2 = 0.72$.

Overestimation of the carotid wall thickness by CTA compared with histologic examination likely resulted from the carotid endarterectomy specimen including only the intima and part of the media, whereas CTA imaging considered the entire carotid wall. The same reason may explain why some plaques that were classified as type 0 or 1 to 2 on histologic examination were classified as 1 to 2 or 3 by CTA. A similar issue was previously reported for MR imaging of the carotid plaques.29

Table 3: Comparison of CTA classification and gold standard histologic classification of type of atherosclerotic plaque and stage of lesion development according to the system derived from the American Heart Association (AHA) classification system

<table>
<thead>
<tr>
<th>Histologic Classification</th>
<th>Type of Plaque</th>
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<th>I–II</th>
<th>III</th>
<th>IV–Va</th>
<th>Vb</th>
<th>Vc</th>
<th>Vla</th>
<th>Vlb</th>
<th>Vlc</th>
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<td>III</td>
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Table 4: Comparison of CTA and gold standard histologic examination for large calcifications (Vb plaques)

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<th>Large Calcifications (Vb Lesions)</th>
<th>Histologic Examination</th>
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<td>CTA</td>
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Table 5: Comparison of CTA and gold standard histologic examination for small and large lipid cores (III, IV–Va, Vb plaques)

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<th>Small and Large Lipid Cores (III, IV–Va, Vb Lesions)</th>
<th>Histologic Examination</th>
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<td>CTA</td>
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Table 6: Comparison of CTA and gold standard histologic examination for large lipid cores only (IV–Va plaques)

<table>
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<th>Large Lipid Cores (IV–Va Lesions)</th>
<th>Histologic Examination</th>
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<td>CTA</td>
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Note:—CTA indicates CT angiography.

CTA did not perform well in classifying all lipid cores ($\kappa = 0.495; P = .492$) (Table 5), likely because the overlap in Hounsfield densities for connective tissues and lipids makes it difficult to distinguish small lipid cores. However, when only large lipid cores (≥5 pixels, IV–Va plaques) are considered, CTA classified lesions in greater concordance compared with histologic examination ($\kappa = 0.796; P < .001$) (Table 6). The 5 pixels cutpoint was determined by a sensitivity analysis showing that, when 5 pixels or more fall into a class of HU, the specificity in characterizing correctly the corresponding histologic component was superior to 90%.

CTA had similar difficulty in classifying all hemorrhages but again improved when only large hemorrhages (≥5 pixels, VIb plaques) were considered ($\kappa = 0.712; P = .102$) (Table 7).

CTA also showed strong concordance with histologic features when classifying ulcerations (Vla plaques), which resulted in a $\kappa$ of 0.855 (Table 8).
An alternative explanation would be some degree of shrinkage during the histologic preparation.

**Discussion**

This study provides proof of principle that the composition of atherosclerotic plaques determined by CTA accurately reflects composition of the lesion as defined by histologic examination. To the best of our knowledge, this is the first report on the use of a CTA-derived, automated classification computer algorithm to distinguish the “type” of each image pixel (connective tissue, lipid-rich necrotic core, hemorrhage, and calcifications) located within the carotid wall. We found that the CT-derived algorithm was excellent in classifying calcifications. CTA classification worked less well for classifying lipid-rich necrotic cores and hemorrhage, likely because the range of densities associated with these components overlaps with the densities associated with connective tissue. This overlap severely limited the reliability of individual pixel Hounsfield readings to indicate fibrous tissue, hemorrhage, or lipid-rich necrotic cores; it posed less of a limitation when larger areas of lesion were considered. Indeed, CTA classification showed good correlation with histologic examination when only large lipid cores and large hemorrhages were considered. CTA classification also performed well in detecting ulcerations and in measuring the fibrous cap thickness.

The results of our study are in agreement with previous studies that used CT to characterize atherosclerotic plaque in carotid,31,32,35–37,40 popliteal,39 or coronary38,39,41 arteries. This was most notable with regard to identifying calcifications,36–39 and concerning identifying attenuation ranges for the different plaque components.31,32,40 Our study also supports previous findings that CT can play a role in the identification of carotid plaque ulcerations.30 In contrast to our study, previous studies did not use a systematic approach to identify all components of carotid atherosclerotic plaque but either adopted a qualitative approach consisting of drawing regions of interest around supposed components,31,39,40 or simply averaged the attenuation for the entire arterial wall seen on a section, without stereologic differentiation.35,37 Previous studies typically focused on only 1 specific plaque component such as calcium, differentiating between calcified and noncalcified plaques, without going further in the characterization of the components.32,36,38,39,41

Our study is unique in several ways. We used modern multidetector-row CT scanners with rapid acquisition, no motion artifact, and better contrast profiles and enhancement, whereas previous studies have reported on older generations of CT scanners (some single-section)31,32,35–40 with relatively thick (3 mm) sections.35,37 In our study patients, we optimized the intraluminal enhancement using a bolus timing strategy to time our CTA acquisition to the early arterial phase. The high intraluminal enhancement that was obtained with this approach increased the accuracy of the segmentation of the inner contour of the carotid artery wall by the software, by reinforcing the contrast between the lumen and the wall. We used an automated, computed analysis rather than an observer interpretation as in previous studies.29 Automated classification algorithms have previously been proposed with MR imag-
ing 25,27,47,48 but not CT. Automated classification algorithms, such as the one presented in our study, could lead to improved reproducibility in characterization of plaques and could be of interest in longitudinal studies of progression of atherosclerotic disease.24,49

Unlike sonography, CT cannot be performed at the bedside, and it has inferior tissue contrast resolution compared with MR imaging. However, CTA of the carotid arteries is a routine imaging test that is frequently obtained as part of the standard of care of patients with acute or chronic cerebrovascular disease. At the present time, interpretation of CTA studies focuses on the degree of luminal narrowing. Our study shows that attention should also be paid to the characteristics of the carotid wall as demonstrated on CTA, because they reflect histologic composition. CTA provides an absolute quantitative measure of tissue composition, whereas signal intensity on MR imaging is only a relative value. This information is included in the dataset obtained as part of the standard-of-care CTA. The potential clinical applications of our results are enhanced by the fact that CTA can be obtained in a few seconds in the clinical setting and does not require any specific research imaging protocol.

Our study had several limitations. We did not correlate the plaque composition with patients’ symptoms because of the limited sample size and that all 8 patients presented with TIsAs. During the quantitative analysis, we did not perform a pixel-by-pixel comparison but, rather, a small square-by-small-square comparison. The resulting heterogeneity of tissue within squares was associated with a corresponding heterogeneity of attenuations and was taken into account by the linear mixed model analysis. After quantitative analysis, we did not calculate the sensitivity, specificity, negative predictive value, positive predictive value, or accuracy of CT for determining the different components. Rather, we evaluated whether the thresholds afforded could characterize the types of plaques, which are more clinically relevant. We did not examine the degree of luminal narrowing on CTA in our study participants because this was examined in a different study.

Finally, because our algorithm and the characterization of the plaques were validated in the same sample in which the thresholds were defined, there was the potential for an overfitting bias. The strong agreement between CTA and histologic classification of plaques could be overly optimistic given this limitation. To address this issue, validation of the algorithm in a different, prospective sample of patients is required.

In conclusion, the composition of carotid atherosclerotic plaques determined by CTA accurately reflects the composition of plaques defined on histologic examination. The ability to analyze components of carotid plaques on CTA with an automated classification algorithm could provide a convenient, repeatable, noninvasive method of studying carotid atherosclerotic disease in longitudinal studies. Correlation of CTA-derived assessment of carotid plaques with symptoms and risk for stroke remains to be investigated.

Acknowledgments

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References


