Relationship between Calcification and Vulnerability of the Carotid Plaques

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Background: Carotid plaques with a high degree of calcification are usually considered at low embolic risk. However, since a precise evaluation of the extent of calcification is not possible preoperatively through duplex ultrasound and postoperatively by conventional histological examination due to the decalcification process, the relationship between the amount of calcium involvement and plaque vulnerability has not been evaluated yet. This study aims to correlate the extent of carotid plaque calcification with clinical, radiological, and histological complications.

Methods: Symptomatic and asymptomatic consecutive patients submitted to carotid endarterectomy between January to December 2014 were included in the study. The amount of carotid calcification was assessed at preoperative computed tomography (CT) through measurement of thickness and circumferential calcium extension and graded from 1 to 8 accordingly (Babiarz classification). Patients were then categorized into 2 groups (low-level group: grade 1–5; high-level group: grade 6–8) and correlated with clinical characteristics and ipsilateral cerebral ischemic lesions at CT. Vulnerability of the plaque was assessed histologically according with American Heart Association (AHA) Classification. Results were overall blindly correlated.

Results: One hundred five patients (81% male; age: 73 ± 8 years) were enrolled in the study. Forty (38%) were symptomatic and 43 (40%) had an ipsilateral focal lesion at preoperative cerebral CT. Thirty-six (38%) patients had high-level carotid calcification degree at CT scan. At histological analysis, 56 (56%) plaques were considered complicated (AHA type VI). Patients with high-level and low-level carotid calcification had similar epidemiological risk factors, preoperative neurological symptoms, and histological complications (17% vs. 15%, P = 0.76 and 50% vs. 55%, P = 0.62, respectively). The high-level calcification group showed a significantly higher incidence of ipsilateral cerebral lesions at preoperative CT (56% vs. 32%, P = 0.01).

Conclusions: A high level of calcification of the carotid plaque is not necessarily associated with lower vulnerability: the incidence of preoperative neurological symptoms and histological complications is similar in patients with and without extensive carotid plaque calcification. Cerebral ischemic lesions may be even more frequent in the presence of highly calcified plaques.

INTRODUCTION

Large randomized, controlled trials have demonstrated the benefit of carotid endarterectomy (CEA) in patients with hemodynamically significant stenosis of the bifurcation. Nevertheless, since most asymptomatic patients remain stroke-free with the sole medical therapy, many authors suggest carotid...
revascularization only when elements of vulnerability are evident in the carotid plaque.\textsuperscript{1} As a matter of fact, morphological characteristics of the plaque may be more important than the degree of stenosis in the prediction of future strokes, thus identification of plaques at high embolic risk may improve treatment strategies.\textsuperscript{2}

Calcification is a prominent structural feature of advanced atherosclerotic plaques and is readily detected with spiral computed tomography (CT).\textsuperscript{3} Most studies have focused on the mechanisms involved in calcification of the coronary arteries.\textsuperscript{4} and the correlation of the arterial wall calcification with plaque extension.\textsuperscript{3} However, the role of calcification in plaque stability remains unclear. Although B-mode ultrasound is being increasingly used to identify rupture-prone unstable plaque, characterization of overall plaque calcification is imprecise with this technique.\textsuperscript{6,7}

Although definite histopathologic features, such as a large lipid-rich necrotic core with thin fibrous cap, extensive macrophage infiltration, and paucity of smooth muscle cells\textsuperscript{8} are associated with vulnerability, the role of calcification is less clear.

The purpose of our study was therefore to quantify the carotid artery bifurcation calcification in symptomatic and asymptomatic patients by using multidetector row CT and determine its relationship with clinical symptoms and histopathologic features of plaque instability.

**METHODS**

**Study Design and Setting**

Symptomatic and asymptomatic consecutive patients undergoing CEA between January to December 2014, at an academic center, were prospectively entered in the study. The inclusion criteria were the availability of a computed tomography (CT) angiography of the neck and head performed no more than 1 month before the carotid intervention and the histological suitability of the atherosclerotic carotid specimens. The exclusion criteria were nonatherosclerotic plaques, restenosis, and previous radiotherapy of the neck or head. All patients gave their informed consent for the procedure. The study was performed according to the rules of the Ethical Review Board of our institution.

The primary end point was to compare the clinical characteristics and histological elements of vulnerability (histological complications) in high and low calcified carotid plaque at preoperative CT.

**Patients**

Carotid revascularization was performed for asymptomatic (>60%) or symptomatic (>50%) carotid artery stenosis (according to the North American Symptomatic Carotid Endarterectomy Trial Collaborators\textsuperscript{9} criteria) fulfilling the requirements of Society for Vascular Surgery guidelines. The asymptomatic status was defined as absence of any cerebral or retinal events ipsilateral to the carotid stenosis in the past 6 months as in the Asymptomatic Carotid Surgery Trial (ACST).\textsuperscript{10} By clinical investigation, in cases of doubts, an adjunctive evaluation by in-hospital neurologist was obtained. Clinical characteristics, technical aspects, and perioperative (30-day) outcome were entered into a dedicated database as previously described.\textsuperscript{11,12} The clinical characteristics included the following: age; sex; hypertension; dyslipidemia; diabetes mellitus; current smoking; coronary artery disease; chronic obstructive pulmonary disease; and chronic renal failure (glomerular filtration rate <60 mL/min). Perioperative medical therapy was also considered, specifically antiplatelet therapy, statins, and oral anticoagulant therapy. All patients had a preoperative cerebral CT scan evaluation as a routine standard procedure in our institution before carotid revascularization.\textsuperscript{13,14} CEA was performed according to the Society for Vascular Surgery guidelines.\textsuperscript{15} The CT angiography of the neck and head was at discretions of the physician requests in the preoperative period.

Trained vascular surgeons performed all CEA procedures (under general anesthesia with routine shunting and Dacron patching). The 30-day stroke and death rate were collected.

**Carotid Calcification Evaluation at CT**

The amount of carotid calcification was assessed at preoperative carotid plaque CT, performed at least 1 month before the carotid intervention, through measurement of thickness and circumferential calcium extension and graded from 0 to 4, respectively, accordingly with Babiarz classification.\textsuperscript{16,17} The total amount of calcification (by the sum of the thickness and circumferential scores) ranged from 0 to 8, and patients were then categorized into 2 groups: low-carotid calcification (grade 0–5) and high-carotid calcification (grade 6–8), Figure 1.

**Cerebral Ischemic Lesions Evaluation at Computed Tomography**

An expert neuro-radiologist evaluated the cerebral CT scan, as previously reported, and any ischemic damage was classified using the Stevens
were considered for the analysis only the cerebral lesion ipsilateral to the stenosis and considered of embolic origin (type from 1 to 4):

1. (Embolic) Discrete subcortical was small, well-circumscribed hypodense lesion, usually only a little greater than 1 cm in size, adjacent to apparently noninvolved cerebral cortex in the anterior and middle cerebral artery territory.

2. (Embolic) Large cortical infarcts had cortical distribution occupying usually >50% of the entire anterior and middle cerebral artery territories.

3. (Embolic) Small cortical infarcts had cortical distribution occupying usually substantially <50% of the entire anterior and middle cerebral artery territories.

4. (Embolic) Basal ganglia infarcts were 1 or more circumscribed lesions in the basal ganglia or thalami, usually about 1 cm.

5. (Nonembolic) Watershed infarction involved cortical and subcortical regions at the periphery of the middle cerebral artery supply territory.

6. (Nonembolic) Diffused white matter low-density changes were areas of poorly circumscribed low density in the cerebral white matter, usually bilaterally.

7. (Nonembolic) Lacunar state was multiple hypodensities in the basal ganglia and thalami, bilaterally.

**Histological Carotid Plaque Evaluation**

Histologic analysis was performed as previously reported. Carotid plaques were removed in full during surgery to preserve the plaque structure. Decalcification (up to 6 hours) was applied if requested, samples were cut in serial sections, and the area with the highest percentage of stenosis was identified and defined as the area of interest for further analysis. Plaque tissue samples were fixed in formalin buffered 4% and embedded in paraffin; 5-μm-thick hematoxylin and eosin-stained sections were observed under a light microscope (LM, Olympus CX42). Carotid atherosclerotic lesions were defined according to the original American Heart Association (AHA) classification of 1995 and grouped as type I-VIII. Histological features examined were lipid core, neoangiogenesis and inflammation (scored from 0 to 5), and the maximum and minimum thickness of the fibrous cap. In according to AHA classification, a vulnerable and rupture-prone plaque was definite as AHA type VI, complicated with hemorrhage, surface defects, and/or thrombus elements. An experienced pathologist performed all histopathological analysis.

**Statistical Analysis**

Continuous data are expressed as mean ± standard deviation (SD) and categorical variables by relative and absolute frequencies. Analysis of differences between groups was performed with \( \chi^2 \) test for categorical variables and with unpaired Student’s \( t \)-test for continuous variables. Linear regression was performed to evaluate a possible correlation between the values of histological features and the calcification score at carotid CT. The sample size evaluation was performed to speculate a possible clinical benefit from the identification of calcific plaque as a protective factor from plaque vulnerability: with the hypothesis of 10% of vulnerability in “high
calcific'' plaques and 40% of vulnerability in “low calcific’’ plaques, the whole sample—for an alpha error of 5% and beta error of 20%—was calculated in 80 specimens. The value of $P < 0.05$ was considered significant. Statistical tests were performed using Statistical Package for Social Sciences (SPSS 22.0) for Windows computer software (SPSS, Chicago, IL).

RESULTS

Clinical Characteristics and CT Calcification Evaluation

In a 1-year period, of 208 CEA procedures performed, 105 patients met the inclusion criteria for the present study. No postoperative complication (stroke or death) occurred in the present population. General clinical characteristics are reported in Table I; in particular, 80% of patients were male, and the mean age of all population was 73 ± 8 years; 40 (38%) were symptomatic, stroke and TIA, and 42 (40%) had an ipsilateral preoperative cerebral ischemic lesion at CT-scan.

The calcium total score evaluated in the whole population was 4.5 ± 2.5, and 36 (34%) patients were considered at high-level carotid calcification (calcium total score ≥ 6).

The presence of high calcific plaque was not significantly associated with clinical characteristics or medical therapies. In the neurological status evaluation, the high-calcific plaque was not associated with preoperative symptoms (TIA or stroke), and time elapsed from surgery but the presence of preoperative ipsilateral cerebral ischemic lesion was significantly associated with the presence of high-calcific plaque (48% vs. 32%, $P = 0.01$), Table I.

Histological Characteristics and CT Calcification

All the 105 specimens were suitable for the histological analysis. Table II showed the distribution of histological plaque characteristics. In particular, 56 (53%) were considered complicated plaques (AHA type VI). The evaluation by linear regression of the carotid calcium total score by CT and the histological elements was associated with a trend to inverse correlation with lipid core extension (Figs. 2 and 3). No correlation was identified between the histological elements and the high- and low-calcific plaques, with the exception of the lipid core that was less extended in the high-calcific plaques compared with low-calcific plaques (1.1 ± 1.3 vs. 2.1 ± 1.1, $P = 0.01$, respectively), Table II. The distribution of histological complications (grade VI of AHA
classification) was similar between high- and low-calcific plaques (50% vs. 55%, $P = 0.62$, respectively).

**DISCUSSION**

This study aimed to correlate the extent of carotid plaque calcification with clinical, radiological, and histological complications. For that reason, we used CT angiography to quantify the degree of carotid plaque calcification in 105 symptomatic and asymptomatic consecutive patients submitted to carotid endarterectomy. The importance of the calcification scoring by the CT is due to the high sensibility of this technique to calcium; moreover, the evaluation of calcification by CT imaging allows a more accurate histological evaluation of the element of plaque vulnerability because of the method of carotid specimens processing needs a decalcification phase that not enable to a correct calcification evaluation. The presence of high-calcific plaque was not significantly associated with preoperative neurological symptoms or medical therapies. The distribution of histological complications (grade VI of AHA classification) was similar between high- and low-calcific plaques (50% vs. 55%, $P = 0.62$, respectively).

**Table II.** Histological characteristics of carotid plaques and comparison between high and low CT calcium score patients

<table>
<thead>
<tr>
<th>Histological characteristics</th>
<th>Total (105), %</th>
<th>High CT calcium % ($n = 36$)</th>
<th>Low CT calcium % ($n = 69$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHA VI plaque</td>
<td>53 (56)</td>
<td>50 (18)</td>
<td>55 (38)</td>
<td>0.62</td>
</tr>
<tr>
<td>Maximum fibrous cap, μm</td>
<td>1,061 ± 398</td>
<td>1,190 ± 325</td>
<td>1,001 ± 417</td>
<td>0.06</td>
</tr>
<tr>
<td>Minimum fibrous cap, μm</td>
<td>235 ± 196</td>
<td>304 ± 262</td>
<td>230 ± 155</td>
<td>0.14</td>
</tr>
<tr>
<td>Lipid core</td>
<td>1.8 ± 1.4</td>
<td>1.1 ± 1.3</td>
<td>2.1 ± 1.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Inflammation</td>
<td>1.6 ± 1.3</td>
<td>1.4 ± 1.2</td>
<td>1.7 ± 1.4</td>
<td>0.20</td>
</tr>
<tr>
<td>Neoangiogenesis</td>
<td>1.2 ± 1.2</td>
<td>1.0 ± 1.2</td>
<td>1.3 ± 1.1</td>
<td>0.20</td>
</tr>
</tbody>
</table>

AHA, American heart association.

Calcification is frequently encountered in atherosclerotic disease and has been proposed as a stabilizing factor rather than a risk factor for carotid plaque instability by some authors; however, the results of the present study and of other authors lead to different conclusions. The relevance of atherosclerotic plaque calcification to plaque structural stability has been investigated most commonly in the coronary arteries. Coronary calcific deposits detected with CT appear to be associated with an increased risk of coronary events. However, the relationship between the amount of calcium involvement and plaque vulnerability has not been extensively evaluated yet in the carotid plaque. Kwee reported that clinically symptomatic plaques have a lower degree of calcification than asymptomatic plaques; however, his systematic review was biased by a significant level of heterogeneity in the calcification evaluation. Shaalan et al. observed a strong inverse correlation between the extent of carotid plaque calcification and the intensity of plaque fibrous cap inflammation as determined by the degree of macrophage infiltration and proposed the spiral CT as a quantitative marker for cerebrovascular ischemic event risk.

Differently from these experiences, some other authors failed to identify an element of plaque stabilization in the carotid calcification, similarly to the present work. Collett and Canfield analyzed the correlation of calcification with neoangiogenesis within the plaque, finding a direct correlation between spot calcification and microvascularization. Those authors suggested that angiogenic factors can influence the bone and cartilage formation through the cytokines released by endothelial cells, which can induce the differentiation of osteoprogenitor cells. New et al. in the analysis of 136 carotid plaques correlated the amount of calcification with the presence of macrophage. Similarly, Eisenmenger et al. investigated the grade of carotid calcification by CT angiography and identified a high correlation with intraplaque hemorrhage. Other authors reported data from the Rotterdam study. Van den Bouwhuijsen et al. analyzed 329 carotid specimens and identified a correlation between carotid calcification and intraplaque hemorrhage; the authors concluded that different types of calcification are possible, and some of them are more frequently associated with plaque hemorrhage compared with others. Selwaness et al. by evaluating 1731 carotid specimens found no correlation between the extent of plaque calcification and neurological symptoms, similarly to the present work. Moreover, in the evaluation of 2 large atherosclerotic carotid plaque biopsy bank, involving more than 1,600 carotid plaques,
no relationship between carotid calcification and new cerebral symptoms was found.\textsuperscript{30}

Giving all these premises, a high level of carotid calcification cannot be considered an element of plaque stability. Our work showed another interesting result, such as a correlation between highly calcified plaques and presence of cerebral ischemic lesion at CT (56\% vs. 32\%, $P = 0.01$), which is known to be associated with high risk for cerebral event in patients with carotid stenosis.\textsuperscript{31}

We can only speculate that the calcification process within the plaque can be determined in 2 different ways. In the first one, there can be an evolution of an intraplaque hematoma determining a vulnerable plaque; in the second case, the calcification is caused by the macrophage osteoblastic activity, which leads to a more stable plaque.

The present study suffers from some limits. First, the population studied is heterogeneous, with both symptomatic and asymptomatic patients enrolled; however, this heterogeneity can be useful to compare the specimens according to the previous neurological symptoms and to consider the clinical vulnerability of the plaque. Next, many of carotid revascularizations were performed after 2 weeks from symptoms; consequently, the preoperative CT scan data may not be precisely representative of the actual carotid specimens.

In conclusion, a high level of carotid plaque calcification is not necessarily associated with a low grade of vulnerability; as a matter of fact, the incidence of preoperative neurological symptoms and histological vulnerability is similar in patients with and without extensive carotid plaque calcification. At present, the simple evaluation of the extension of carotid plaque calcification cannot be considered a valid element to stratify the risk of vulnerability.

**Fig. 2.** Linear regression analysis of the calcium total score evaluated by computed tomography and lipid core score (A), inflammation score (B), and neoangiogenesis score (C).

**Fig. 3.** Linear regression analysis of the calcium total score evaluated by computed tomography and minimum (A) and maximum (B) thickens of the fibrous cap.
REFERENCES


