Eur J Vasc Endovasc Surg **32**, 561–567 (2006)
doi:10.1016/j.ejvs.2006.02.019, available online at http://www.sciencedirect.com on ScienceDirect

Quantification and Characterization of Carotid Calcium with Multi-detector CT-angiography

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Objective. *The aim of this study was to assess the accuracy of CT-angiography for identification and measurement of calcification of carotid atherosclerotic plaques and to characterise the content and distribution pattern of mineral calcium (hydroxyapatite, Ca) in carotid bifurcations and investigate its relationship with neurological symptoms.*
Methods. *Twenty-six patients with ICA stenosis >60% (13 symptomatic, 13 asymptomatic) were selected for study. Ca was estimated from the weight of the ashed remnants of carotid endarterectomy (CEA) specimens in 11 patients. Calcium content (calcification volume (mm^3),CV), and average calcium density (Hounsfield units (HU),CD), were determined by CT-angiography. The distribution pattern of calcium within the lesion (base (posterior), shoulder or luminal surface) was assessed in all cases.*
Results. *CT-derived estimation of CV and Ca mass (modified Agatston Score, (mAS) = CV × CD) showed a good correlation with its direct measurement in CEA specimens ($r = 0.911$ and 0.993 respectively, $p < 0{,}005$). Asymptomatic patients with ICA stenosis >60% showed statistically significant higher content of Ca than those who were symptomatic (mAS: 122.6 ± 138.0 HU mm^3 vs 42.8 ± 59.1 HU mm^3, $p = 0.04$). Calcification on the surface of the plaque was observed more commonly in asymptomatic patients (9/12 vs 3/15, $p = 0.006$). Non-calcified or plaques with posterior calcification were 12 times more likely to be symptomatic (OR: 12, 95%CI 1.5–91.1, $p = 0.021$).*
Conclusions. *CT-angiography permits the reliable quantification of calcification of carotid plaques. A lower content of calcium in carotid plaques, as well as its distribution in the base of the lesion, was associated with a greater prevalence of neurological symptoms. These parameters may be useful to identify those patients at higher risk of stroke.*

Keywords: Carotid; Calcification; Risk factors; Stroke; Computed tomography.

Introduction

A relationship between calcification of the atherosclerotic plaque and the subsequent risk of developing cardiovascular events has been suggested in several studies.[1,2] However, the lack of a reliable method for *in vivo* detecting and quantifying calcium in the arterial wall has precluded evaluation of calcium content in cross-sectional and follow-up studies. Calcification of atherosclerotic plaques has been considered to be a major drawback for imaging carotid artery, being responsible for the posterior shadowing in duplex scanning and artifacts that preclude correct interpretation of standard angiograms. Calcification is not resolved on magnetic resonance imaging (MRI). Only recently, electron-beam computed tomography (EBCT) and multi-detector computed tomography (MDCT)) have permitted the accurate identification and measurement of mineral calcium (hydroxyapatite, Ca) in atherosclerotic plaques.

Several scoring systems have been suggested for CT-based estimation of calcification from serial sections (Agatston score), or based on 3D volumetric reconstructions with multiple detectors or helical CT. The accuracy of these scoring methods and their relationship with the subsequent risk of developing coronary ischaemic events has been assessed.[3,4] However, there is less information about reliability and predictive value of assessing the risk of stroke associated with atherosclerotic lesions of the extracranial carotid artery.

The aim of this study of carotid arteries was: (i) To assess the accuracy of CT-angiography to identify and measure calcification of atherosclerotic plaques; (ii) To characterise the content and distribution pattern of calcification at the carotid bifurcation and investigate its relationship with neurological symptoms.

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1078–5884/000561 + 07 $35.00/0

Methods

Thirty two consecutive patients undergoing CT-angiography to evaluate stenosis of the internal carotid artery (ICA > 60%), were included. Nineteen patients were symptomatic (stroke: 12; TIA: 2; ocular symptoms: 5), and 13 were asymptomatic. Exclusion criteria included: age > 80 years old (limit for CEA by protocol); previous ipsilateral CEA; non-specific or vertebro-basilar symptoms. Twenty-six patients had ICA stenosis of >60%, 13 symptomatic and 13 asymptomatic patients and were selected for further investigations, in a study approved by the local ethical review board.

All the patients underwent CT exam under angiography protocol in a 4-MDCT scanner (Somaton Sensation 4, Siemens). Helical acquisition sequences from C6 to C2 were obtained. Scan parameters included: 1.25 mm nominal section thickness; 1.5:1 pitching; 12 cm field of view; 120 mL of non-ionic contrast agent was injected at 4 mL/s. Image data were subsequently transferred to a computer workstation (Workstation Advantage Windows 4.2, GE Healthcare) for post-processing. Semi-transparent 3D reconstructions (2 cm proximally and 1 cm distally to carotid bifurcation) were generated for volume analysis (Fig. 1).

Calcium content (calcification volume (mm^3),CV); average Ca density (Hounsfield units (HU),CD; and modified Agatston Score (mAS = CV × CD))) were determined in those patients with ICA stenosis >60% (Fig. 2). A threshold of CD = 420 HU was used to differentiate calcifications from intra-vascular contrast agent.

Ca quantification was determined from CEA specimens, by ashing the samples at 700 °C during 72 hours, in 11 patients. Ca content was estimated from the weight of the remnants (96% hydroxyapatite). These results, and the corresponding CT-based measurements, were used to design a linear regression equation in order to normalise CT results.

Distribution pattern of Ca within the lesion was assessed in all cases by two independent observers unaware of patient clinical status. They were qualitatively classified as: 1. Non-calcified; 2. Posterior (located at the base of the lesion); 3. Shoulder (identified in the marginal angles). 4. Superficial (placed on the luminal surface).

Other variables (demographic, clinical and laboratory data) are summarised in Table 1.

Statistical analysis

Simple linear regression analysis was performed to compare Ca content, as estimated from ashed CEA specimens, and CT-derived measurements.

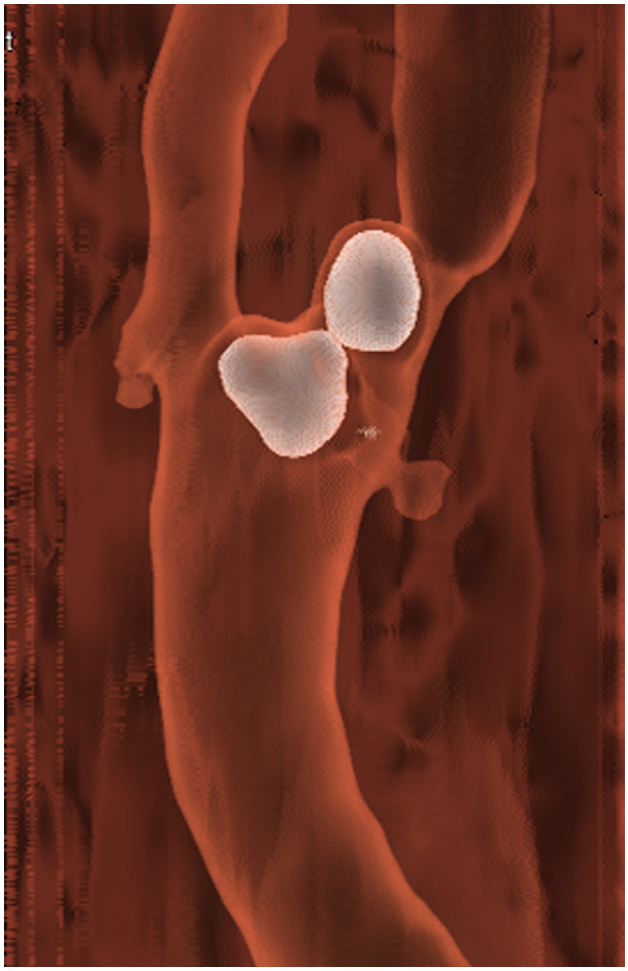

Fig. 1. Semitransparent 3D projection of CT angiography showing two calcifications at the carotid bifurcation. A threshold attenuation of 420 HU was used to differentiate mineral calcium from intravascular contrast.

Non-parametric U Mann-Whitney test was used to compare the means of Ca content in both groups (symptomatic and asymptomatic). chi^2 test was used for comparison of intra-plaque Ca distribution.

Logistic regression analysis was performed to assess the Odds ratios of calcification parameters, risk factors and laboratory data. The presence or absence of cerebral ischaemic symptoms was considered as dependent variable. A p value of less than 0.05 was considered to be statistically significant.

Results

There were no differences between symptomatic (n = 13) and asymptomatic (n = 13) patients, except for calcification parameters (Ca density and mAS), total cholesterol (higher in symptomatic patients) and

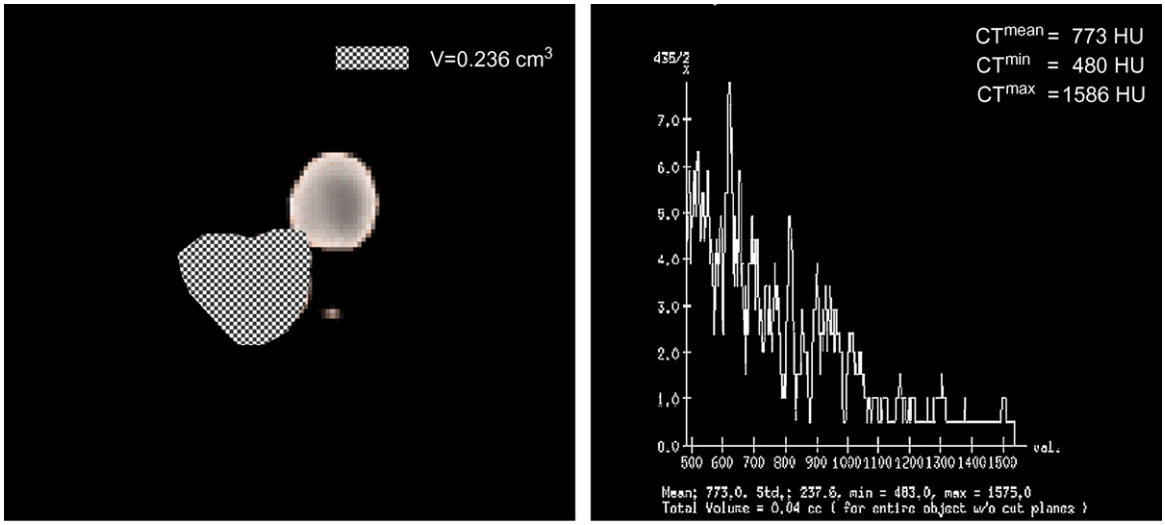

Fig. 2. Calculation of modified Agatston Score (mAS) as the product of calcification volume (left), and average attenuation (CTmean) (right).

hypertension (more prevalent in asymptomatic patients) among classical risk factors. These data are summarised in Table 1.

CT accuracy for quantification of plaque Ca

CT-derived estimation of CV and Ca content (modified Agatston Score, (mAS) = CV × CD) showed a good correlation with its direct measurement in CEA specimens (r = 0.911 and 0.993, respectively, $p < 0.005$). Linear regression analysis showed a good correlation between Ca quantification from ashed CEA specimens and CT-based measurements. Regression plots and equations for volume and Ca mass extrapolated from mAS are shown in Fig. 3. These data indicate that CT angiography predicted 83% and 98.7% (R^2) of the variability observed in the direct measurement of Ca mass and volume respectively.

Table 1. Demographic, clinical, and laboratory data in symptomatic and asymptomatic patients

Variable	Symptomatic (n = 13) n (%)	Asymptomatic (n = 13) n (%)	*p*
Hypertension	8 (61.5)	13 (100)	***0.04***
Smoking	7 (53.8)	5 (38.5)	0.53
Diabetes	5 (38.5)	6 (46.2)	0.69
Dyslipidemia	7 (53.8)	8 (61.5)	0.69
Coronary disease	6 (46.2)	4 (30.8)	0.42

Variable	Symptomatic mean (SD)	Asymptomatic mean (SD)	*p*
Leucocytes ($\times 10^3/mm^3$)	7.46 (2.32)	7.04 (1.76)	0.60
Glucose (mmol/l)	7.27 (2.54)	7.58 (2.91)	0.77
total cholesterol (mmol/l)	5.08 (0.99)	3.43 (0.93)	***0.04***
LDL cholesterol (mmol/l)	2.91 (0.32)	2.8 (0.76)	0.79
Triglycerides (mmol/l)	1,92 (0.82)	1.36 (0.65)	0.08
Calcium (mg/dl)	9.32 (0.41)	9.13 (0.47)	0.86
Ionic calcium (mg/dl)	1.32 (0.04)	1.31 (0.04)	0.17
Phosphorus (mg/dl)	3.68 (0.42)	3.51 (0.74)	0.48
PTH (I.U.)	77.50 (61.39)	39.19 (20.81)	0.12
Vitamin D (I.U.)	13.48 (5.01)	23.83 (13.39)	0.92
Plaque Volume (mm^3)	63.38 (80.84)	150.92 (156.88)	0.07
Plaque Density (HU)	401.92 (359.92)	686.92 (234.76)	***0.04***
mAS (HU $mm^3 \times 10^3$)	42.83 (59.14)	122.63 (137.97)	***0.04***

Content and distribution of plaque Ca

CT-angiography identified 26 patients with ICA stenosis >60%. Thirteen were symptomatic and 13 asymptomatic. CT did not confirm ICA stenosis >60% in 6 further patients, who were excluded. In asymptomatic patients, the carotid artery with most severe stenosis was considered for comparison.

Post-processing of CT images detected calcification of carotid bifurcation in 23 carotid bifurcations. Ca distribution within the plaque of selected patients is shown in Fig. 4.

CV (mm^3) was lower in symptomatic patients than in asymptomatic ones (63.8 mm^3 vs 150.9 mm^3), but this difference did not reach statistical significance. Symptomatic patients had a significantly lower mAS than asymptomatic patients (42.8 ± 59.1 HU mm^3 vs 122.6 ± 138.0 HU mm^3, $p = 0.04$) (Fig. 5 left). Calcification in the luminal surface and shoulders of the plaque was found significantly more frequently in asymptomatic than in symptomatic patients (9/12 (75%) vs 3/15(13.3%), $p = 0.006$). (Fig. 5 right). Poor

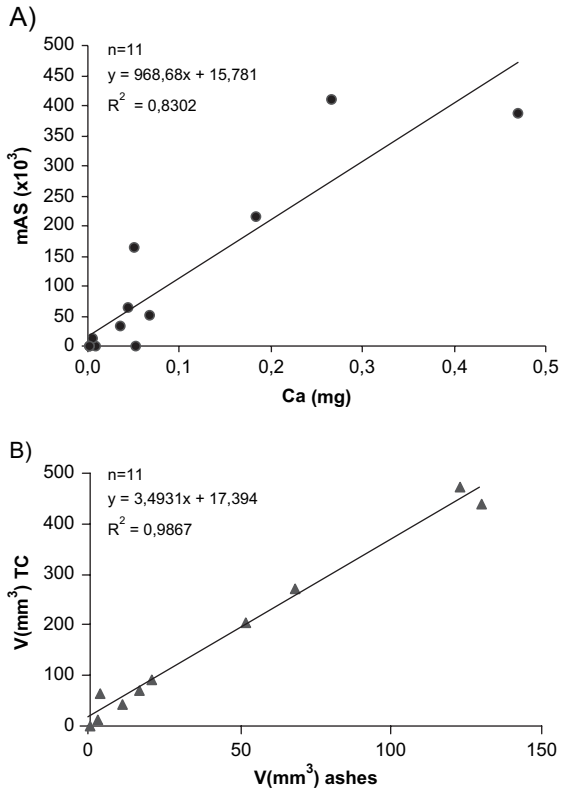

Fig. 3. Regression plot comparing the results of calcification mass (A) and volume (B), measured from remnants after ashing of surgical specimens of carotid endarterectomy (X axis), and CT-derived measurements (Y axis).

quality precluded the assessment of Ca distribution within the plaque in 5 patients.

Multivariate analysis

Intra-plaque Ca distribution, Ca content, hypertension and cholesterol plasma levels initially were identified as potentially significant variables for logistic regression analysis. However, only Ca distribution was accepted in the model. Its odds ratio and 95% confidence interval is shown in Table 2, to indicate that, in this study, non-calcified plaques, and those with posterior calcification, showed a 12-fold increased risk of being symptomatic (OR: 12, 95%CI 1.5–91.1, $p = 0.021$). The regression equation correctly classified 77.3% of cases. The large confidence interval was probably due to the small size of the sample. Therefore, caution is advisable before extrapolating these results to the general population.

Discussion

The relationship between the stenosis degree of the internal carotid artery (CAS) and the risk of stroke has been suggested in the randomise trials of symptomatic patients *(North American Symptomatic Carotid Endarterectomy Trial (NASCET 1991)*,[5] and *European Carotid Symptomatic Surgery Trial (ECST))*,[6] demonstrating the effectiveness of CEA in the most severe degrees of stenosis (>70%). However, its relationship is less evident in moderate stenosis (CAS = 31–70%), or even in severe but asymptomatic stenosis, suggesting the necessity to consider other features in the carotid plaque apart from degree of stenosis. The search for biological markers, or imaging parameters related to plaque composition, in order to define subpopulations at high risk of stroke, has been one of the main targets of vascular medicine in the last decade.

Characterisation of atherosclerotic plaque with MRI techniques (2D-spin echo, T2-weighted, and 3D-gradient echo) has been discussed.[7] High intensity signals from collagenous cap are well differentiated from the necrotic core at sub-millimetre resolution in 3D reconstructions, providing relevant information on plaque architecture. Bassiouny *et al.*[8] have related cap thickness to the risk of stroke (500 μm in asymptomatic patients (n = 40) and close to 300 μm in symptomatic ones (n = 59)). However, the presence of calcification in atherosclerotic plaque results in strongly decreased signal intensity, especially in T2 weighted sequences, leading to artifacts secondary to distortion of the magnetic field. Previous studies have demonstrated the accuracy of CT measuring of calcium content in surgical specimens of carotid endarterectomy (CEA), by comparison with the residual mass after ashing of the samples (corresponding to 96% hydroxyapatite; r = 0.99).[9]

The rationale for a potential protective role of arterial calcification against thromboembolic events, and the subsequent risk of stroke, is based on histopathology studies that show a lower Ca content of CEA specimens of symptomatic patients.[10] This evidence has been further reinforced by the subjective estimation of carotid calcification with duplex-scanning in series of symptomatic and asymptomatic patients.[11,12]

More recently, multi-detector computed tomography (MDCT) has demonstrated its reliability to quantitatively confirm this relationship *in vivo*. Nandalur *et al.* semi-quantitatively classified atherosclerotic carotid plaques from 31 patients with stenosis >60% (15 symptomatic and 21 asymptomatic). Calcified plaques were 21 times less likely to be symptomatic than non-calcified ones[10] In the present study, asymptomatic patients also had a significantly higher plaque Ca content than asymptomatic ones (ASm: 122.6 ± 138.0 HU mm^3 vs 42.8 ± 59.1 HU mm^3, $p < 0.05$) and calcified plaques on the luminal surface were 12 times less likely to have been symptomatic. No

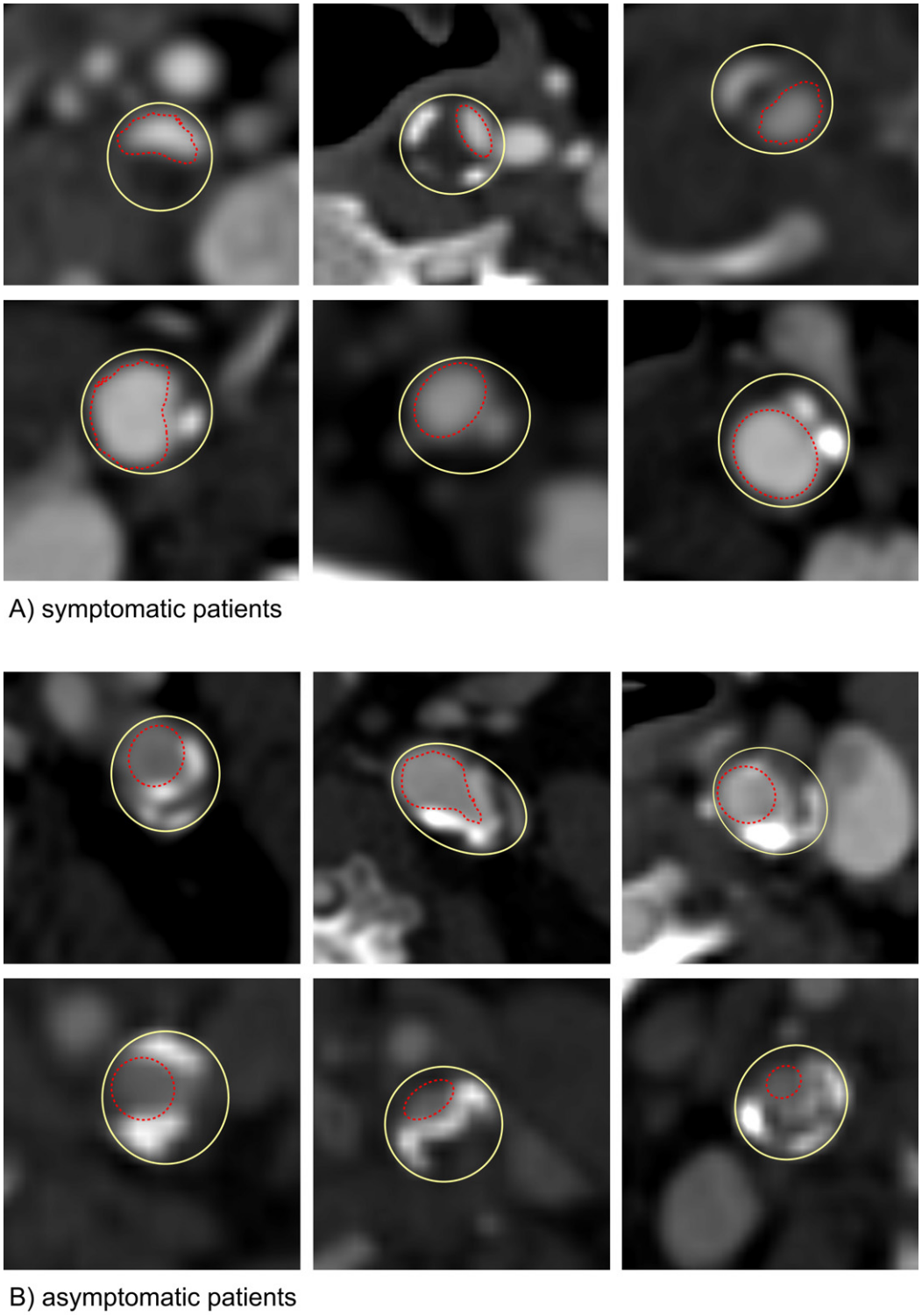

Fig. 4. Distribution of calcifications in carotid plaques from selected symptomatic (A), and asymptomatic patients (B). Mineral calcium is preferentially located at the base or within the plaque in the former, and at the surface or shoulders in the latter.

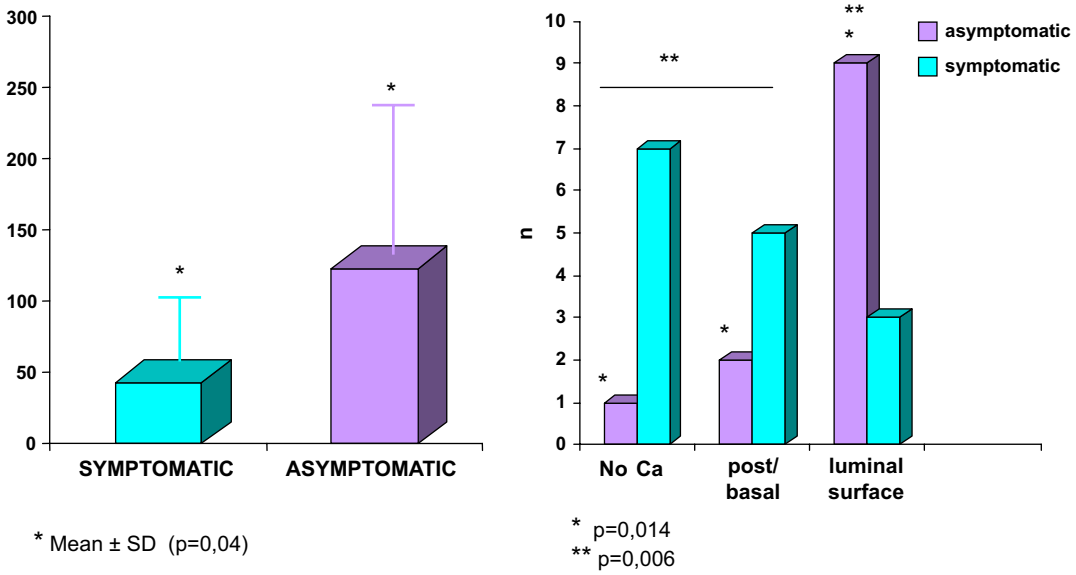

Fig. 5. Average modified Agatston Score (Asm) (left), and distribution of calcifications within the plaque (right), in asymptomatic and symptomatic patients.

other independent variable (demographic, clinical or laboratory data) was predictive of neurological symptoms. This again suggests the potential role of Ca parameters as a marker of the risk of stroke, perhaps with a more important role than classical risk factors for prediction of cardiovascular events.

Although CT is probably the most reliable method for *in vivo* quantification of calcification of the atherosclerotic plaque, it has some major drawbacks. First, resolution limit is dependent on the voxel size in 3D reconstruction. Second the "Partial volume effect" overestimates the measurement of radiological structures as a result of assigning a complete voxel despite its partial occupancy in the periphery of the image. Third, the overlapping of densities corresponding to intra-vascular contrast and Ca precludes correct identification of low density calcifications.

A CT attenuation threshold of 130 HU has been suggested to resolve Ca from surrounding tissues. However, this limit may result too low in presence of a contrast agent. This feature has been specifically analysed by Estes *et al.*[13] Characterization of atherosclerotic plaque with CTangiography revealed that calcium is recognized with an attenuation value of 350−500 HU, while intra-luminal contrast required setting the threshold at 150−300 HU and fibrous tissue and lipids were identified at 90 ± 24 HU and 39 ± 12 HU, respectively. In the present study a CT attenuation threshold of 420 HU was considered to differentiate calcifications from intra-vascular contrast. This high limit may lead to underestimate true calcification volumes.

In conclusion, CT-angiography permits the reliable quantification of calcification of atherosclerotic plaques. In this study, a lower content of calcium in carotid plaques, as well as its distribution in the base of the lesion, was associated with a greater prevalence of neurological symptoms. These parameters may be useful to identify those patients at higher risk of stroke.

Table 2. Predictive variables accepted in logistic regression model to identify symptomatic and asymptomatic patients

	B	Odds ratio	95%CI	*p*
Constant	−3.989			0.019
Intra-plaque Ca distribution[a]	2.49	12	(1.58−91.09)	0.021

[a] Absent or localised at the base of the lesion.

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Accepted 1 February 2006
Available online 18 September 2006