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# Atherosclerosis and Dementia

## A Cross-Sectional Study With Pathological Analysis of the Carotid Arteries

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**Background and Purpose**—Previous ultrasound-based studies have shown an association between carotid artery atherosclerosis and dementia. Our aim was to investigate this association using postmortem examination.

**Methods**—Postmortem morphometric measurements of carotid stenosis and intima-media thickness were performed in individuals with dementia (n=112) and control subjects (n=577). Multivariate logistic regression models were applied.

**Results**—High-grade left internal carotid stenosis ( $\geq 70\%$ ) was associated with increased odds for dementia (OR, 2.30; 95% CI, 1.14–4.74;  $P=0.02$ ). Intima-media thickness was not associated with dementia.

**Conclusions**—The likelihood of dementia is increased with high-grade left internal carotid artery atherosclerosis after adjusting for demographic and cardiovascular risk factors. (*Stroke*. 2011;42:3614-3615.)

**Key Words:** carotid stenosis ■ dementia ■ pathology

Previous studies using ultrasound as an assessment method have shown an association between carotid artery atherosclerosis (CAA), including intima-media thickness (IMT), and a higher risk of dementia.<sup>1–3</sup> Although ultrasound is a well-established method in routine clinical practice, it is associated with interexaminer variability in IMT and plaque measurements.<sup>4</sup> Moreover, specific segments of the carotid artery are difficult to evaluate, particularly the bulb and the internal carotid artery.<sup>5</sup> Morphometric evaluation of CAA in autopsy specimens allows evaluation of the limits of the plaque along the entire arterial length and the precise definition of the boundaries of the intima and media layers. We aimed to investigate the association of dementia and CAA measured by morphometric methods in a large autopsy series.

### Methods

Specimens were collected from 2005 to 2008 at the Human Brain Bank of the Brazilian Aging Brain Study Group, including cases >50 years of age from a general autopsy service in São Paulo, Brazil. Demographics, cardiovascular risk factors, and cognitive status were obtained with the next of kin after signing a statement of informed consent approved by the local ethics and research committee. See <http://stroke.ahajournals.org> for complete methodology in the supplemental section.

The percentage of carotid stenosis was calculated by subtracting the lumen area from the intima area, dividing the difference by the intima area, and multiplying the result by 100 (Figure). IMT was

calculated by dividing the intima-media area (defined as the area internal to the external elastic lamina up to the lumen) by the media perimeter (delineated by the external elastic lamina).

Neuropathological examination was performed in a subgroup of participants. Microvascular changes were analyzed semiquantitatively using hematoxylin and eosin staining in 14 routinely sampled areas plus additional areas detected at the macroscopic examination. The presence of lacunae, microinfarcts, and infarcts was registered. Clinical and neuropathological evaluations were carried out double-blinded.

We conducted univariate and multivariate logistic regression analyses to assess the correlation between dementia (binary outcome) and carotid artery obstruction (defined as of  $\geq 70\%$  obstruction) and quartiles of IMT. Results were adjusted to demographic, cardiovascular risk factors, and presence of stroke.

### Results

A total of 689 cases were analyzed. Demographics and cardiovascular risk factors of the sample are shown in the Table. Stroke reported by the family was more prevalent among demented individuals (25.0% versus 8.8%;  $P<0.001$ ). Of the 689 participants, 233 (33.4%) had neuropathological examination. Lacunar infarctions were present in 32 participants (13.7%) and was similarly distributed between individuals with and without dementia (14 of 71 versus 18 of 162,  $P=0.08$ , respectively). High-grade stenosis was detected in 3% to 9.4% of participants according to the artery segment (Supplemental Table I). The mean IMT was  $1.12\pm 0.30$  mm for the common carotid artery and  $0.86\pm 0.34$  mm for the internal carotid artery.

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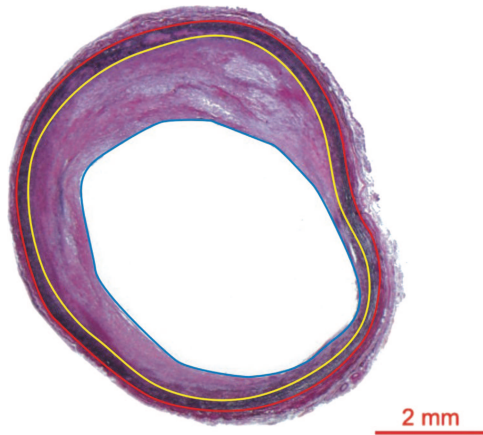
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**Figure.** Histological representation of the common carotid artery using Verhoeff stain. Red indicates external elastic lamina; yellow, internal elastic lamina; blue, lumen. Calibration bar=2 mm.

In the multivariate model adjusting for potential confounding factors, we observed that high-grade left internal carotid stenosis is associated with increased odds for dementia (OR, 2.55; 95% CI, 1.26–5.15;  $P=0.009$ ). This association remains significant even after additional adjustment for the presence of stroke ( $P=0.02$ ). There was a nonsignificant trend for an association between right internal carotid artery stenosis and dementia (OR, 1.96; 95% CI, 0.94–4.08;  $P=0.07$ ). Stenosis located at the common carotid arteries was not associated with dementia (left: OR, 1.76; 95% CI, 0.67–4.61;  $P=0.25$  and right: OR, 0.24; 95% CI, 0.02–2.52;  $P=0.23$ ; Supplemental Table I). Results are similar when selecting only the moderate and severe cases of dementia versus no dementia. Individuals with and without dementia were

**Table. Demographics and Cardiovascular Risk Factors From Participants With and Without Dementia (n=689)**

Variable	Dementia	No Dementia	P
Age, y, mean (SD)*	80.0 (9.3)	68.7 (11.2)	<0.001
Male gender, no. (%)†	39 (34.8)	319 (55.3)	<0.001
White, no. (%)†	82 (73.2)	386 (67.5)	0.23
Education, y, mean (SD)*	3.1 (3.0)	4.5 (3.7)	<0.001
Hypertension, no. (%)†	64 (57.1)	372 (64.5)	0.14
Diabetes, no. (%)†	34 (30.4)	161 (27.9)	0.60
Dyslipidemia, no. (%)†	6 (5.4)	56 (9.7)	0.14
Coronary artery disease, no. (%)†	21 (18.8)	164 (28.4)	0.04
Heart failure, no. (%)†	12 (10.7)	115 (19.9)	0.02
Stroke, no. (%)†	28 (25.0)	51 (8.8)	<0.001
Physical inactivity, no. (%)†	94 (83.9)	324 (56.2)	<0.001
Smoking, no. (%)†	15 (13.4)	159 (27.6)	<0.01
Drinking, no. (%)†	17 (15.2)	147 (25.5)	0.02
Body mass index, m/kg <sup>2</sup> , mean (SD)*	21.1 (3.8)	23.7 (4.4)	<0.001
Total, no. (%)	112 (16.3)	577 (83.7)	

SD indicates standard deviation.

\*Mann-Whitney U test.

† $\chi^2$  test.

similar for IMT (common: OR, 0.94; 95% CI, 0.79–1.13;  $P=0.52$  and internal: OR, 0.87; 95% CI, 0.73–1.05;  $P=0.14$ ).

## Discussion

Although widely used, the interpretation of ultrasonographic evaluation may be dependent on sonographer expertise and can be difficult to perform due to unfavorable patient biotype. In the Rotterdam Study, common carotid artery IMT was measurable in 96% of individuals, carotid bulb in 64%, and internal carotid artery in 31%.<sup>5</sup> Our study is the first to describe the association between dementia and CAA using morphometric evaluation in autopsy specimens.

We found differences that were specific for side and segment of the carotid artery segment. Severe left-sided atherosclerotic lesions are expected to have a greater impact on cognition than CAA on the right side.<sup>1</sup> Differences in hemodynamics among distinct carotid artery segments may explain why the association between CAA and dementia is significant only for severe stenosis located in the internal carotid artery. In our study, IMT showed no association with dementia. Previous studies have reported conflicting results regarding this association.<sup>1–3</sup>

Cognitive impairment may be associated with high-grade stenosis of the internal carotid artery even without evidence of infarction on MRI or a history of stroke.<sup>1</sup> Indeed, in our study, the analysis of 233 patients showed that prevalence of lacunar infarctions is similar between individuals with and without dementia. Cerebral small vessel diseases may mediate the link between atherosclerosis and dementia through direct vascular brain damage or by accelerating clinical expression of dementia. Silent embolization and chronic cerebral hypoperfusion are also possible mechanisms implicated in CAA-related cognitive impairment. Because we did not assess language and aphasia may impact the dementia diagnosis, our results need to be interpreted in light of this limitation. Further studies, including animal models, are needed to clarify the precise etiology of the dementia and to determine the impact of vascular brain lesions on cognitive function.

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## Disclosures

None.

## References

- Johnston SC, O'Meara ES, Manolio TA, Lefkowitz D, O'Leary DH, Goldstein S, et al. Cognitive impairment and decline are associated with carotid artery disease in patients without clinically evident cerebrovascular disease. *Ann Intern Med*. 2004;140:237–247.
- van Oijen A, de Jong FJ, Witteman JCM, Hofman A, Koudstaal PJ, Breteler MMB. Atherosclerosis and risk for dementia. *Ann Neurol*. 2007; 61:403–410.
- Romero JR, Beiser A, Seshadri S, Benjamin EJ, Polak JF, Vasan RS, et al. Carotid artery atherosclerosis, MRI indices of brain ischemia, aging, and cognitive impairment: The Framingham Study. *Stroke*. 2009;40:1590–1596.
- Schulte-Altdorneburg G, Droste DW, Kollár J, Beyna T, Felszeghy S, Módis L, et al. Accuracy of in vivo carotid B-mode ultrasound compared with pathological analysis: intima-media thickening, lumen diameter, and cross-sectional area. *Stroke*. 2001;32:1520–1524.
- del Sol AI, Moons KG, Hollander M, Hofman A, Koudstaal PJ, Grobbee DE, et al. Is carotid intima-media thickness useful in cardiovascular disease risk assessment? The Rotterdam Study. *Stroke*. 2001;32:1532–1538.

## **SUPPLEMENTAL MATERIAL**

### **Supplemental Methods**

#### *Participants and post mortem cognitive evaluation*

In the present study, specimens collected at the Human Brain Bank of the Brazilian Aging Brain Study Group (HBB-BABSG) were used, including cases older than 50 years of age from a general autopsy service located in Sao Paulo, Brazil [1]. The HBB-BABSG holds a large sample of brains from non-demented subjects and individuals suffering from mild to severe dementia. For inclusion in the study, next-of-kin agreed to participation by signing a statement of informed consent previously approved by local ethics and research committee. Exclusion criteria of the brain bank are: (a) death from primary cerebral disease in which there was a need to elucidate the cause of death during the autopsy, (b) potential cerebral ischemic lesions secondary causes of chronic hypoperfusion not related to carotid stenosis (e.g. severe chronic obstructive pulmonary disease) which could compromise cognitive function prior to death by interfering in brain homeostasis and (c) cerebrospinal fluid acidosis due to a terminal condition. Specimens collected from 2005 to 2008 were analyzed for this study. The demographics and cardiovascular risk factor profile were obtained through a structured interview with the next-of-kin who had contact with the patient at least once a week during the six months prior to death.

Cognitive status was assessed post-mortem using information provided during the clinical interview with the next-of-kin. The Clinical Dementia Rating (CDR) scale was used to evaluate cognitive function [2]. Participants were clinically classified for the presence of dementia and its severity (without dementia = CDR 0; mild dementia =

CDR 1; moderate dementia = CDR 2; and severe dementia = CDR 3). Individuals with questionable dementia (CDR=0.5) were excluded. To further support the diagnosis of dementia, the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) scale was used [3]. A cut-off value of 3.4 was adopted to discriminate between individuals with and without cognitive impairment. Individuals rated with  $CDR \geq 1$  and  $IQCODE \geq 3.4$  were considered demented. The control group comprised subjects with  $CDR=0$  and  $IQCODE < 3.4$ .

*Anatomic post-mortem assessment of carotid artery atherosclerosis, intima-media thickness and neuropathological evaluation*

Carotid arteries were conserved in 70% ethyl alcohol and later filled with agar to prevent artery collapse. Subsequently, the specimens were fixed in 10% paraformaldehyde and then cut into 5 mm-thick segments. The segments with the greatest obstruction were selected from among the common and internal carotid arteries (right and left sides), embedded in paraffin and cut into 8- $\mu$ m thick sections. Each of these regions was stained using Verhoeff's stain and photographed with a stereomicroscope (Nikon® SMZ 1000). The image processing program ImageJ® was used for measuring the lumen and intima areas. The intima area was delineated from the internal elastic lamina up to the endothelial surface. The percentage of carotid stenosis was calculated by subtracting the lumen area from the intima area, dividing the difference by the intima area and then multiplying the result by 100 (Figure 1).

IMT was calculated by dividing the intima-media area (defined as the area internal to the external elastic lamina up to the lumen) by the media perimeter (delineated by the external elastic lamina). This measure represents an estimate of the

mean IMT of the whole wall of the carotid artery slice. The result was stratified by quartiles of IMT of the common and internal carotid artery, and compared for the presence of dementia.

Neuropathological examination was performed in a subgroup of participants. Microvascular changes were analyzed semi-quantitatively using H&E staining in 14 routinely sampled areas plus additional areas detected at the macroscopic examination [1]. The presence of lacunae, microinfarcts, and infarcts were registered. Clinical and neuropathological evaluations were carried out double blinded.

#### *Statistical analysis*

The main dependent variable was presence of dementia (binary outcome). We initially conducted univariate analysis assessing the correlation between dementia and carotid artery obstruction (obstruction was defined as of greater or equal than 70% obstruction). We also conducted univariate analyses with other variables such as quartiles of carotid artery IMT. The next step was to conduct multivariate logistic regression analyses in order to adjust the results for the presence of confounders, such as demographic and cardiovascular risk factors. Demographic factors selected for adjustment *a priori* were age at death, gender and years of education. Cardiovascular risk factors selected *a priori* were history of hypertension, diabetes, dyslipidemia, coronary artery disease, heart failure, physical inactivity, smoking and alcoholism, according to the clinical interview. Finally, the results were further adjusted for the presence of stroke. The level of significance of all tests was set at 5% in 2-sided tests. The statistical analyses were performed using the Stata statistical software version 10.0 (Stata Corp., College Station, Texas).

**Supplemental Table – Crude and adjusted OR and 95% CI for the association between dementia and carotid artery stenosis (n=689)**

	<b>Dementia</b>		<b>Crude OR</b>	<b>Model 1*</b>	<b>Model 2**</b>
	<b>Yes</b>	<b>No</b>	<b>(95%CI)</b>	<b>OR (95%CI)</b>	<b>OR (95%CI)</b>
<b>RCCA stenosis, n(%)</b>					
< 70%			1	1	1
≥ 70%	111 (99.1)	557 (96.5)	0.25 (0.03-1.89)	0.39 (0.05-3.27)	0.24 (0.02-2.52)
<b>p-value</b>	1 (0.9)	20 (3.5)	0.18	0.39	0.23
<b>RICA stenosis, n(%)</b>					
< 70%			1	1	1
≥ 70%	96 (85.7)	528 (91.5)	1.80 (0.98-3.29)	1.86 (0.92-3.80)	1.96 (0.94-4.08)
<b>p-value</b>	16 (14.3)	49 (8.5)	0.06	0.09	0.07
<b>LCCA stenosis, n(%)</b>					
< 70%			1	1	1
≥ 70%	104 (92.9)	542 (93.9)	1.19 (0.54-2.64)	1.99 (0.78-5.05)	1.76 (0.67-4.61)
<b>p-value</b>	8 (7.1)	35 (6.1)	0.67	0.15	0.25
<b>LICA stenosis, n(%)</b>					
< 70%			1	1	1
≥ 70%	94 (83.9)	531 (92.0)	2.21 (1.23-3.98)	2.55 (1.26-5.15)	2.30 (1.11-4.74)
<b>p-value</b>	18 (16.1)	46 (8.0)	0.008	0.009	0.02
<b>Total, n(%)</b>	112 (16.3)	577 (83.7)			

RCCA=right common carotid artery; RICA=right internal carotid artery; LCCA=left common carotid artery; LICA=left internal carotid artery.

Logistic regression models

\*Model 1: Adjusted for age, gender, years of education, hypertension, diabetes, dyslipidemia, coronary artery disease, heart failure, physical inactivity, smoking, drinking and body mass index.

\*\*Model 2: Model 1 plus stroke.

## Supplemental References

1. Grinberg LT, Ferretti REL, Farfel JM, Leite R, Pasqualucci CA, Rosemberg S, Nitri R, Saldiva PHN, Jacob-Filho W for Brazilian Aging Brain Group. Brain bank of the Brazilian Aging Study Group – a milestone reached and more than 1,600 collected brains. *Cell Tissue Banking* 2007;8:151-162.
2. Morris JC. The Clinical Dementia Rating Scale (CDR): current version and scoring rules. *Neurology* 1993;43:2412-2413.
3. Jorm AF. The Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE): a review. *Int Psychogeriatr* 2004;16:275-293.