Predominance of Nonatherosclerotic Internal Elastic Lamina Calcification in the Intracranial Internal Carotid Artery

Annelotte Vos, MD; Wim Van Hecke, MD; Wim G.M. Spliet, MD; Roel Goldschmeding, MD, PhD; Ivana Isgum, PhD; Remko Kockelkoren, MD; Ronald L.A.W. Bleys, MD, PhD; Willem P.T.M. Mali, MD, PhD; Pim A. de Jong, MD, PhD; Aryan Vink, MD, PhD

Background and Purpose—Calcification of the intracranial internal carotid artery (iICA) is an independent risk factor for stroke. These calcifications are generally seen as manifestation of atherosclerosis, but histological investigations are limited. The aim of this study is to determine whether calcifications in the iICA are present in atherosclerotic plaques, or in other parts of the arterial wall.

Methods—Thirty-nine iICAs were histologically assessed, using digital microscopy to quantify the amount of calcification in the different layers of the arterial wall.

Results—Calcifications were found in the intima, around the internal elastic lamina and in the medial layer of the arterial wall. In 71% of the arteries, internal elastic lamina calcification contributed most to the total calcified cross-sectional surface area. Internal elastic lamina calcification was unrelated to the occurrence of atherosclerotic intimal lesions. Intimal calcifications were most often associated with atherosclerotic lesions, but also many noncalcified atherosclerotic lesions were found.

Conclusions—In the iICA, calcifications are predominantly present around the internal elastic lamina, suggesting that this nonatherosclerotic type of calcification contributes to the previously observed increased risk of stroke in patients with iICA calcifications. (Stroke. 2016;47:221-223. DOI: 10.1161/STROKEAHA.115.011196.)

Key Words: atherosclerosis  humans  internal carotid artery  stroke  vascular calcification

Calcifications of the intracranial internal carotid artery (iICA), diagnosed using computed tomographic (CT) scans, are an independent risk factor for stroke that contributes to the occurrence of 75% of all strokes. The presence of calcified atherosclerotic plaques seems to be a logical explanation for the observed association between calcification and stroke. However, older literature describes the occurrence of both intimal and medial calcification in the siphon of the ICA, the latter being composed of calcification of both media and internal elastic lamina (IEL) of the arterial wall. If medial/IEL calcification is regularly present in the iICA, the calcification on imaging may not be a proxy for atherosclerosis, and nonatherosclerotic calcification might also contribute to the previously described risk of stroke. The aim of this study is to describe the amount, location, and morphology of calcifications in the iICA.

Methods

The iICA was examined in 20 consecutive patients (39 arteries; 20 left and 19 right) administered for cerebral autopsy in our hospital. Material was handled according to the code of proper use of human tissue, used in The Netherlands. Collection of the material was approved by the local biobank review committee (protocol 15–252). The ICA was dissected close to the circle of Willis and the petrous bone. The arteries were fixed in 4% formaldehyde, and subsequently decalcified using EDTA. Decalcification was necessary to maintain the morphology, and interpretability, of the arterial wall layers. Because histological evaluation of calcification is based on visualization of matrix previously altered by the calcification process, and not calcium ions itself, decalcification does not influence analysis (Methods section in the online-only Data Supplement). To study atherosclerosis that affects arteries heterogeneously, the arteries were divided in a proximal and distal segment. Microscopic slides of rings cut perpendicularly to the lumen were stained with hematoxylin and eosin and elastin van Giesson.

Digitalized slides (Scan-Scope XT scanner; Aperio Technologies) were analyzed using Aperio Image Scope software. Calcifications were identified as sharply demarcated, acellular spots, and areas (Figure I in the online-only Data Supplement). Intimal calcification was, arbitrarily, considered substantial when ≥1% of the intimal surface was calcified.

Because of right-skewed distributions, χ² tests were performed. Missing data were only because of technical issues. Available case analyses were performed. Data are presented as median and interquartile range, unless specified otherwise.

Received August 27, 2015; final revision received September 25, 2015; accepted September 29, 2015.


The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.115.011196/-/DC1.

Correspondence to Aryan Vink, MD, PhD, Department of Pathology, University Medical Center Utrecht, Room H.04.3.12, PO Box 85500, 3508 GA Utrecht, The Netherlands. E-mail a.vink@umcutrecht.nl

© 2015 American Heart Association, Inc.

Strok e is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.115.011196
Results
Median age of the patients was 64 years (range, 41–90 years). In 16 patients, a recent cerebral CT scan was available. The iICA calcifications present in 15 of 16 patients were comparable with those presented in literature. Histologically, calcifications were observed at 3 locations: in the intima, around the IEL, and in the media.

Intimal Calcification
Substantial intimal calcification was found in 27 of 78 arterial segments. In these 27 segments, atherosclerotic lesions were more often present (85%) than nonatherosclerotic lesions (15%; \( P < 0.0005 \)). The other way around, however, in atherosclerotic lesions (37/78 arterial segments), substantial calcification was found in only 62% (\( P = 0.139 \); Table I in the online-only Data Supplement).

IEL Calcification
Thirty-eight of thirty-nine arteries showed calcification of (part of) the circumference of the IEL (Figure 1). The median percentage of the circumference that was calcified was 36% (26% to 45%). Sixty-four percent of the arteries had a maximal calcification of >50% of the circumference, which was not related to the occurrence of atherosclerotic lesions or intimal calcification. In 22 of 78 arterial segments, >50% of the IEL circumference was calcified, with in 10/22 coexistence with an atherosclerotic intimal lesion (\( P = 0.670 \)) and in 7 of 22 with substantial intimal calcification (\( P = 0.09 \)).

Medial Calcification
Calcification in the medial layer was only found in small amounts (Figure II in the online-only Data Supplement).

Total Arterial Wall Calcification
Thirty-eight of thirty-nine arteries revealed substantial calcification of intima (≥1% intimal surface area calcified) or IEL (≥1% of circumference calcified) or media (≥1% medial surface area calcified). IEL calcification contributed most to the total calcified cross-sectional surface area (79%; 47%–95%), followed by intimal (14%; 0%–51%) and medial calcification (0%; 0%–4%; Figure 2; Table II in the online-only Data Supplement). Furthermore, in 71% of the arteries with substantial calcification of intima or IEL or media, the absolute surface of IEL calcification was biggest, followed by intimal (26%) and medial (3%) calcification.

Discussion
Calcification of the iICA on CT scan was recently identified as an independent risk factor for stroke.\(^1\) The present study has 2 important results. First, we found that iICA calcifications are present not only in the intimal layer of the arterial wall but also around the IEL and in the media. More importantly, in the majority of arteries (71%) the contribution of IEL calcification to the total calcified cross-sectional surface area in the vascular wall is biggest. These results suggest that also IEL calcification is detected when iICA calcification is measured by CT scanning and IEL calcifications might, therefore, be at least partly responsible for the previously observed association between iICA calcification and stroke. Second, although intimal calcification is related to the presence of atherosclerotic plaques, IEL calcification is not.

IEL calcifications might be a plausible risk factor for stroke because consequent stiffening of the arterial wall will lead to increased pulse pressure,\(^6\) which leads to microvascularity and tissue damage.\(^7\) Furthermore, a relation
between increased pulse pressure and cerebrovascular events has been suggested. It would also be possible that reduced capacity to expand the vascular wall of stiffened arteries could lead to increased mechanical stress on atherosclerotic plaques, increasing the risk of plaque rupture and stroke.

Medial and IEL calcifications are potentially reversible lesions, as evidenced by drug-induced (and even spontaneous) regression observed in several other vascular territories. Thus, confirmation of a contribution of IEL/medial calcification to the occurrence of stroke might imply important novel options for stroke risk management.

This study has some limitations. We studied a small cohort of random cerebral autopsy patients, lacking the regular stroke patients. Therefore, these results have to be confirmed in a group of patients with stroke. Furthermore, we only studied the iICA in these patients and, therefore, we cannot relate our findings to atherosclerosis elsewhere in the intracranial or extracranial vasculature.

In conclusion, in the iICA calcifications are predominantly located around the IEL, indicating that these nonatherosclerotic calcifications might contribute to the previously observed increased risk of stroke in patients with iICA calcifications on CT scan.

**Sources of Funding**
The research was supported by a grant of the Netherlands Organization for Scientific Research/Foundation for Technological Sciences (Project 12726).

**Disclosures**
Dr Isgum reports grants from Pie Medical Imaging and The Netherlands Organization for Health Research and Development outside the work described in the article. The other authors report no conflicts.

**References**
Predominance of Nonatherosclerotic Internal Elastic Lamina Calcification in the Intracranial Internal Carotid Artery

*Stroke*. 2016;47:221-223; originally published online October 29, 2015;
doi: 10.1161/STROKEAHA.115.011196

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/47/1/221

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2015/11/03/STROKEAHA.115.011196.DC1
http://stroke.ahajournals.org/content/suppl/2016/12/20/STROKEAHA.115.011196.DC2

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/
Online Supplement

This data supplement has been provided by the authors to give the readers additional information about their work.


Table of contents

<table>
<thead>
<tr>
<th>Content</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplemental methods</td>
<td>2</td>
</tr>
<tr>
<td>Figure I. Examples of intimal calcification</td>
<td>3</td>
</tr>
<tr>
<td>Figure II. Example of medial calcification</td>
<td>4</td>
</tr>
<tr>
<td>Figure III: Pilot study - arterial wall calcification before and after decalcification</td>
<td>5</td>
</tr>
<tr>
<td>Table I. Relation between atherosclerotic lesions and the occurrence of calcification in the intimal layer of the arterial wall</td>
<td>6</td>
</tr>
<tr>
<td>Table II. Contributions of calcifications in the different layers of the arterial wall to the total calcified cross-sectional surface area</td>
<td>7</td>
</tr>
<tr>
<td>References</td>
<td>8</td>
</tr>
</tbody>
</table>
Supplemental methods

After dissecting the intracranial internal carotid artery, the arteries were fixed in 4% formaldehyde for at least 24 hours, followed by decalcification for at least 48 hours using diaminooethylene tetraacetic solution (EDTA) at a concentration of 125 grams per liter of water. This resulted in (partial) decalcification of the specimens, which makes it possible to section the tissue without disrupting the morphology of the vascular wall.

The approach of assessing calcification on decalcified tissue has already been validated.¹ A small pilot study performed in our own center, comparing pieces of non-decalcified arteries and adjacent pieces of decalcified arteries, gave similar results. (Figure III)
Supplemental figures

Figure I. Examples of intimal calcification

A, scattered intimal calcification (^
) seen as purple colored, demarcated dots. B, larger amounts of intimal calcification creating demarcated dark pink to purple clumps.
Figure II. Example of medial calcification

A, presence of calcification (^) at the boundary between media and adventitia. B, Elastin van Giessen stain, showing presence of elastic fibers at the boundary between media and adventitia.

In the figure the different layers of the arterial wall are marked as (i) intima, (IEL) internal elastic lamina, (m) media, and (a) adventitia.

In 24/39 arteries small amounts of medial calcification (range <0.1-5.1% of the medial surface) were present. Only in 4 arteries ≥1% of the total medial surface was calcified.

A notable finding is the presence of calcifications at the boundary of medial and adventitial layer in 12/24 (50%) arteries with medial calcifications. This is the location where, in extracranial arteries, the external elastic lamina is found. Elastin van Giesson (EvG) stains revealed presence of patchy distributed condensation of elastic fibers at the boundary between medial and adventitial layer of the vessel wall.
Figure III: pilot study - arterial wall calcification before and after decalcification

A. The piece of vascular wall cut without decalcification using a CM3600 XP cryomacrotome (Leica Biosystems). The calcifications (marked) are seen as purple chunks. The morphology of the tissue is disturbed, the calcifications are crumbled and partly dragged into the lumen of the artery (indicated by ^).

B. The piece of vascular wall adjacent to the piece in A, decalcified before cutting. The calcified area (marked) is seen as a darker pink, sharply demarcated area. Although the area of calcification in A is a bit disturbed due to dragging in to the lumen (indicated by ^), one can appreciate approximately the same shape of area of calcification in A and B.
Supplemental tables

Table I. Relation between atherosclerotic lesions and the occurrence of calcification in the intimal layer of the arterial wall

<table>
<thead>
<tr>
<th></th>
<th>no/minimal intimal calcification (0-1%)</th>
<th>substantial intimal calcification (≥1%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-atherosclerotic intimal lesion</td>
<td>37</td>
<td>4</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>atherosclerotic intimal lesion</td>
<td>14</td>
<td>23</td>
<td>0.139</td>
</tr>
<tr>
<td></td>
<td>p=0.001</td>
<td>p&lt;0.0005</td>
<td></td>
</tr>
</tbody>
</table>

Intimal lesions present on each cross section were classified according to the modified American Heart Association Classification system. These intimal lesions were further grouped into non-atherosclerotic intimal lesions (intimal thickening and intimal xanthoma) and atherosclerotic intimal lesions (pathological intimal thickening, fibrous cap atheroma, and fibrocalcific plaque). Atherosclerotic intimal lesions were found in 37/78 (47%) segments. In all the other arterial segments, without atherosclerotic plaques, non-atherosclerotic intimal lesions were found. A one cell layer thick intima was never found.
Table II. Contribution of calcification in the different layers of the arterial wall to the total calcified cross-sectional surface area

<table>
<thead>
<tr>
<th></th>
<th>Total area calcified (mm²)</th>
<th>Percentage of total calcified cross-sectional surface area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intima</td>
<td>0.043 (0.001 - 0.152)</td>
<td>14 (0 - 51)</td>
</tr>
<tr>
<td>Internal elastic lamina</td>
<td>0.147 (0.069 - 0.257)</td>
<td>79 (47 - 95)</td>
</tr>
<tr>
<td>Media</td>
<td>0.001 (0.000 - 0.008)</td>
<td>0 (0 - 4)</td>
</tr>
</tbody>
</table>

Results are shown as median and interquartile range.
References

頭盖内頸動脈における非アテローム硬化性内弾性板の石灰化
の好発部位

Predominance of Nonatherosclerotic Internal Elastic Lamina Calcification in the Intracranial Internal Carotid Artery

Annelotte Vos, MD; Wim Van Hecke, MD; Wim G.M. Spliet, MD, et al.
Departments of Pathology, University Medical Center, Utrecht, The Netherlands

背景および目的：頭蓋内頸動脈 (iICA) の石灰化は脳卒中の中の独立した危険因子である。iICA の石灰化はアテローム性動脈硬化症の症状として一般的にみられるが、組織学的な調査は少ない。本研究では、動脈硬化プラクまたはその他の動脈壁部分で iICA の石灰化が存在するか否かを検討することを目的とした。

方法：デジタル顕微鏡で 39 の iICA を組織学的に評価し、動脈壁の各層における石灰化の量を計測した。

結果：石灰化は動脈壁の内膜、内弾性板 (IEL) 周辺および中膜層に認められた。動脈の 71% では、全石灰化断面積のほとんどが IEL の石灰化によるものであった。IEL の石灰化はアテローム硬化性内膜病変の発生とは無関係であった。内膜の石灰化の大部分はアテローム性病変を伴っていたが、石灰化していないアテローム硬化性病変もしくは認められた。

結論：iICA の石灰化は IEL の周辺優位に存在することから、iICA に石灰化病変がある患者で認められた脳卒中リスクの増加は、このアテローム硬化を伴わないタイプの石灰化が一因と考えられる。

Stroke. 2016; 47: 221-223. DOI: 10.1161/STROKEAHA.115.011196.

図 1 内弾性板 (IEL) の石灰化。A：IEL の外周の 76% は多かれ少なかれ連続的に石灰化している（四角の部分）。B：石灰化した IEL の細部。A：外膜。I：内膜。M：中膜。

図 2 全石灰化断面積に対する内膜、中膜、IEL の関与。各層の動脈壁 (内膜、内弾性板 (IEL)、中膜) の全石灰化量の割合を表すボックスプロット。