

# Association Between Carotid Atherosclerotic Plaque Calcification and Intraplaque Hemorrhage

## A Magnetic Resonance Imaging Study

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**Objective**—Carotid intraplaque hemorrhage (IPH) is associated with cardiovascular events. Calcification, which frequently accompanies IPH, may play a role in IPH occurrence. In this study, we aimed to investigate the associations between calcification characteristics and IPH in carotid plaques.

**Approach and results**—One hundred seventeen patients with cerebrovascular symptoms and carotid plaques detected by ultrasound were recruited and underwent multicontrast magnetic resonance imaging. Advanced carotid plaques with composition measured by magnetic resonance imaging were included in the analysis. Carotid calcifications were divided into the following categories: surface, mixed, and deep calcification. They were also classified into single and multiple calcifications according to quantity. Logistic regression models utilizing generalized estimating equations were performed to evaluate the relationship between calcification and IPH. Of 117 subjects, 85 with 142 plaques were included in the final analysis, whereas 32 were excluded because of lack of plaque compositions. Of the 142 plaques, 40 (28.2%) had IPH. Plaques with IPH showed greater prevalence of calcification than those without (87.5% versus 55.9%;  $P=0.005$ ). After adjusting for age, low-density lipoprotein, maximum wall thickness, and maximum soft plaque thickness, multiple calcifications (odd ratio, 10.1; 95% confidence interval, 3.3–30.4), surface calcification (odd ratio, 29.4; 95% confidence interval, 4.1–210.8), and mixed calcifications (odd ratio, 27.9; 95% confidence interval, 7.3–107.1) were found to be strongly associated with the presence of IPH (all  $P<0.05$ ).

**Conclusions**—Surface calcification and multiple calcifications in carotid atherosclerotic plaques are independently associated with the presence of IPH, suggesting that both quantity and location of calcification may play important roles in the occurrence of IPH. These findings may provide novel insights for understanding mechanisms of IPH.

**Visual Overview**—An online [visual overview](#) is available for this article. (*Arterioscler Thromb Vasc Biol.* 2017;37:1228–1233. DOI: 10.1161/ATVBAHA.116.308360.)

**Key Words:** atherosclerosis ■ calcification, physiologic ■ carotid artery ■ intraplaque hemorrhage  
■ magnetic resonance imaging

Vulnerable atherosclerotic plaque in carotid arteries is a major cause of cerebrovascular events, such as transient ischemia attack and ischemic stroke. Previous studies have shown that compositional characteristics, particularly intraplaque hemorrhage (IPH) and lipid-rich necrotic core, in carotid artery plaques play a key role in plaque vulnerability.<sup>1–4</sup> In particular, it is increasingly evidenced that IPH is one of the most effective predictors for cardiovascular events<sup>5,6</sup> because of its stimulating plaque progression by a sharp increase of free cholesterol and macrophage infiltration resulting from erythrocyte membranes.<sup>7,8</sup> Furthermore, it has been reported that the presence of IPH and increased IPH volumes lead to significant increase in critical plaque wall stress and strain and subsequently contribute to plaque instability.<sup>9</sup>

To date, the pathogenesis of IPH is still unclear. Most investigators think that the rupture of immature neovessels may be the pathological basis.<sup>8,10</sup> Neovessels can be affected by multiple factors, such as blood pressure, plaque compositions, and hemodynamic parameters in carotid plaques. A previous study has demonstrated a significant association between IPH detected by MPRAGE (magnetization-prepared rapid gradient-echo) and calcification detected by computed tomography angiography.<sup>11</sup> The investigators also found that calcification may play a role in the occurrence of IPH.<sup>12,13</sup> To our knowledge, few studies have been performed investigating the relationship between calcification and IPH. Xu et al<sup>12</sup> have demonstrated that the location and shape of superficial calcifications in carotid plaques were associated with IPH

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## Nonstandard Abbreviations and Acronyms

CI	confidence interval
IPH	intraplaque hemorrhage
MRI	magnetic resonance imaging
OR	odds ratio

using slice-based statistical analysis. A recent study by van den Bouwhuijsen et al<sup>13</sup> reported that carotid plaques with a higher load of calcification volume had more hemorrhagic components. It therefore seems that both location and the size of calcification are correlated with IPH.

The purpose of this study was to determine the associations between IPH and calcification characteristics, such as quantity and location, in carotid atherosclerotic plaques using magnetic resonance imaging (MRI) in vivo.

## Materials and Methods

Materials and Methods are available in the [online-only Data Supplement](#).

## Results

In total, 85 patients (mean age, 60.1±9.9 years) with complex carotid plaque compositions on MR were included in this study, ranging from February 2012 to April 2014. Of all included subjects, 55 (64.7%) were men, 61 (71.8%) had hypertension, 27 (31.8%) had diabetes mellitus, and 67 (78.8%) had hyperlipidemia. The clinical characteristics of this study population are summarized in Table 1.

### Correlation Between IPH and Clinical Risk Factors

In this population, 145 plaques were found in the carotid arteries. Of these 145 plaques, 3 were excluded because of poor image quality (IQ=1). Of the remaining 142 plaques, 114 were detected from 57 subjects in bilateral carotid arteries and 28 were detected from 28 subjects in unilateral carotid arteries. Of the 142 carotid plaques, 40 (28.2%) had IPH and 92 (64.8%) had calcification. Table 2 presents the differences in clinical risk factors between subjects having plaques with and without IPH and calcification. Compared with carotid plaques without IPH, those with IPH were more frequently seen in older subjects (66±9.4 versus 57.8±9.7 years;  $P=0.003$ ). Subjects who had carotid plaques with IPH showed significantly lower levels of total cholesterol (4.3±0.7 versus 4.9±1.0 mmol/L;  $P=0.034$ ) than those who had plaques without IPH. No significant difference was found in other risk factors between subjects with and without IPH (all  $P>0.05$ ).

### Correlation Between Carotid Plaque Calcification and IPH

Compared with carotid plaques without IPH, those with IPH showed greater maximum wall thickness (4.2±1.7 versus 3.0±1.0 mm;  $P<0.001$ ), greater maximum soft plaque thickness (3.2±1.7 versus 2.1±0.9 mm;  $P<0.001$ ), and a higher prevalence of calcification (87.5% versus 55.9%;  $P=0.005$ ). In carotid plaques with IPH, the incidence of multiple calcifications, surface calcification, and mixed calcifications was significantly higher than in plaques without IPH (all  $P<0.05$ ;

**Table 1. Clinical Characteristics of the Study Population (n=85)**

	Mean±SD or n (%)
Sex, male	55 (64.7)
Age, y	60.1±9.9
Smoking	50 (58.8)
BMI, kg/m <sup>2</sup>	25.2±3.1
Hypertension	61 (71.8)
Hyperlipidemia	67 (78.8)
LDL cholesterol, mmol/L	3.1±1.1
HDL cholesterol, mmol/L	1.0±0.4
TC, mmol/L	4.7±1.0
TG, mmol/L	1.6±0.8
Coronary heart disease	14 (16.4)
Diabetes mellitus	27 (31.8)
Statin use	62 (72.9)

BMI indicates body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; and TG, triglyceride.

Table 3). Moreover, carotid plaques with IPH showed a significantly higher prevalence of stenosis >50% (20.0% versus 2.0%;  $P<0.001$ ) and ulceration (15.0% versus 1.0%;  $P=0.002$ ) than those without IPH (Table 3; Figure). However, the difference in incidences of single calcification or deep calcification was not statistically significant between plaques with and without IPH (Table 3).

Table 4 shows the associations between characteristics of calcification and IPH in carotid plaques. In univariate regression analysis, the presence of calcification (odds ratio [OR], 4.4; 95% confidence interval [CI], 1.6–12.5;  $P=0.005$ ), multiple calcifications (OR, 7.6; 95% CI, 2.6–22.1;  $P<0.001$ ), surface calcification (OR, 12.2; 95% CI, 2.9–50.7;  $P=0.002$ ), and mixed calcifications (OR, 12.4; 95% CI, 3.6–42.5;  $P<0.001$ ) were found to be significantly associated with IPH. After adjusting for age and low-density lipoprotein (model 1) and age, low-density lipoprotein, maximum wall thickness, and maximum soft plaque thickness (model 2), the above associations remained statistically significant (all  $P<0.05$ ; Table 4). In contrast, a significant correlation was not found between the presence of single and deep calcifications and IPH (both  $P>0.05$ ; Table 4).

## Discussion

This study investigated relationships between the characteristics of calcification and IPH in carotid plaques of patients with recent neurovascular symptoms. We found that the presence of multiple calcifications, surface calcification, or mixed calcifications were significantly associated with IPH before and after adjusting for confounding factors. Our findings suggest that the quantity and location of calcification may be independent indicators for IPH in carotid atherosclerotic plaques.

In the present study, age and the level of lipoprotein were found to be associated with IPH. These findings are consistent with previous studies.<sup>14,15</sup> In a recent Rotterdam study,<sup>15</sup> investigators found that IPH occurred more frequently at

**Table 2. Comparison of Clinical Risk Factors Between Plaques With and Without IPH and Calcification**

	Mean±SD or n (%)		<i>P</i> Value*	Mean±SD or n (%)		<i>P</i> Value†
	With IPH (n=26)	Without IPH (n=88)		With CA (n=76)	Without CA (n=34)	
Sex (male)	21 (80.8)	50 (56.8)	0.116	47 (60.5)	20 (61.5)	0.951
Age, y	66±9.4	57.8±9.7	0.003	63.4±9.2	55.4±7.5	<0.001
BMI, kg/m <sup>2</sup>	24.7±3.0	25.4±3.2	0.309	25.0±3.2	25.0±3.3	0.767
Smoke	20 (76.9)	63 (71.6)	0.060	44 (57.9)	17 (50.0)	0.935
Hypertension	20 (66.7)	40 (71.4)	0.720	59 (77.6)	24 (70.6)	0.625
Hyperlipidemia	19 (73.1)	73 (82.9)	0.449	60 (78.9)	32 (94.1)	0.113
TC, mmol/L	4.3±0.7	4.9±1.0	0.034	4.8±1.1	4.8±0.9	0.750
HDL, mmol/L	1.1±0.3	1.1±0.3	0.921	1.0±0.2	1.0±0.4	0.994
TG, mmol/L	1.5±0.7	1.7±0.8	0.481	1.6±0.8	1.7±0.8	0.428
LDL, mmol/L	2.6±0.6	3.3±1.1	0.089	3.2±0.9	3.4±1.3	0.412
Statin use	15 (57.7)	66 (75.0)	0.256	58 (76.3)	29 (85.3)	0.329
Diabetes mellitus	7 (26.9)	30 (34.1)	0.684	28 (36.8)	13 (38.2)	0.614
CHD	4 (15.4)	13 (14.8)	0.925	17 (22.3)	2 (5.9)	0.147

BMI indicates body mass index; CA, calcification; CHD, coronary heart disease; HDL, high-density lipoprotein; IPH, intraplaque hemorrhage; LDL, low-density lipoprotein; TC, total cholesterol; and TG, triglyceride.

\*This comparison excluded 14 subjects who had IPH in a single side of bilateral plaques. In the excluded 14 subjects, 28 plaques were detected (single calcification=3; multiple calcifications=20; surface calcification=5; mixed calcifications=9; deep calcification=9).

†This comparison excluded 16 subjects who had CA in a single side of bilateral plaques. In the excluded 16 subjects, 32 plaques were detected (single calcification=8; multiple calcifications=8; surface calcification=2; mixed calcifications=4; deep calcification=10).

older ages (OR per 10 years, 1.8; 95% CI, 1.6–2.1), in men (OR, 2.2; 95% CI, 1.7–2.9), in patients with hypertension (OR, 1.4; 95% CI, 1.1–1.8), and in current smokers (OR, 1.6; 95% CI, 1.2–2.3). In this study, we found that subjects with carotid IPH showed significantly lower levels of lipoprotein than those without IPH. To date, the effects of statins on IPH and its pathogenesis are still unclear. Because carotid plaques with IPH are at high risk of developing neurovascular events, patients with IPH may have a greater chance to receive statin treatment for stabilizing plaques and levels of lipid proteins may thus become lower than those without IPH. In addition, emerging evidence has shown that blood pressure<sup>14,16</sup> and aspirin use<sup>17</sup> may both play important roles in IPH. The findings of our study offer further compelling evidence of the associations between traditional cardiovascular risk factors and IPH. As such, adjusting for these potential confounding factors is necessary to investigating the relationships between IPH and calcification in the present study.

In the present study, surface calcification was found to be associated with IPH in carotid plaques. These findings are in line with previous studies. In a study by Xu et al,<sup>12</sup> marginal superficial calcification was found to be more often accompanied by IPH compared to central calcification (72.9% versus 46.7%; *P*<0.05). However, this independent association was not assessed by adjusting for clinical confounding factors in study by Xu et al.<sup>12</sup> Moreover, in the present study, MPRAGE is the major sequence used to identify IPH, suggesting a greater incidence of IPH detected compared with the study by Xu et al.<sup>12</sup> This is because MPRAGE has been demonstrated to be more sensitive for identification of IPH than of time of flight and T1-weighted imaging.<sup>18</sup> Compared with the previous study, a major strength of our study is that we investigated

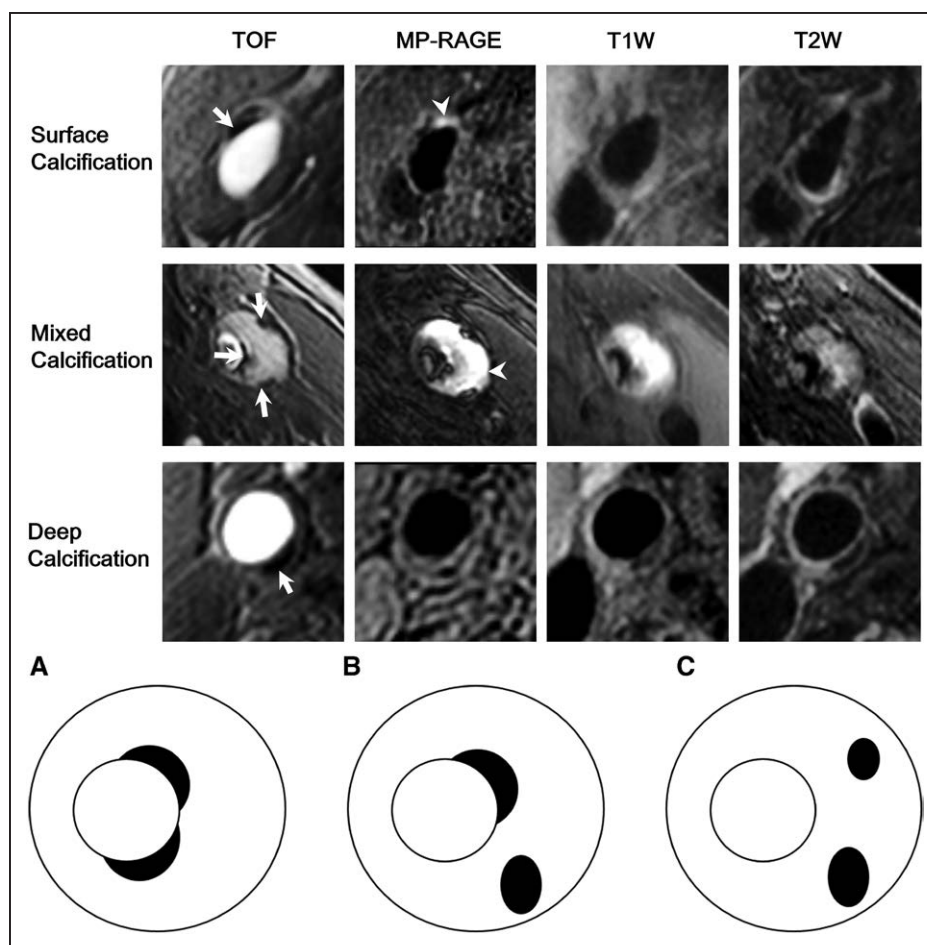
different types of calcification according to their location and quantity rather than surface calcifications alone.

The mechanism of how surface calcification stimulates occurrence of IPH is unclear. Biomechanics may shed light on the relationship between surface calcification and angiogenesis. In a study by Teng et al,<sup>19</sup> maximum local principal stress and

**Table 3. Magnetic Resonance Imaging Characteristics Between Plaques With and Without IPH (n=142)**

	Mean±SD or n (%)		<i>P</i> Value
	With IPH (n=40)	Without IPH (n=102)	
Plaque burden			
Max thickness, mm	4.2±1.7	3.0±1.0	<0.001
Max soft plaque thickness, mm	3.2±1.7	2.1±0.9	<0.001
Stenosis >50%	8 (20.0)	2 (2.0)	<0.001
Ulceration	6 (15.0)	1 (1.0)	0.002
Calcification			
Presence	35 (87.5)	57 (55.9)	0.005
Number			
Single CA	6 (15)	30 (29.4)	0.532
Multiple CA	29 (72.5)	27 (26.5)	<0.001
Location			
Surface CA	10 (25)	6 (6)	<0.001
Mixed CA	20 (50)	14 (13.7)	<0.001
Deep CA	5 (12.5)	37 (36.2)	0.715

CA indicates calcification; and IPH, intraplaque hemorrhage.



**Figure.** Examples of different types of calcification on magnetic resonance (MR) imaging. **Top:** a plaque with surface calcification (arrow) and intraplaque hemorrhage (IPH; arrow head). **Middle:** mixed calcifications (arrow) and IPH (arrow head). **Bottom:** a plaque with deep calcification (arrow) but no IPH. Surface calcification was located within or very close to its cap but did not have fibrous tissue completely covering it. In contrast, deep calcification was located within the plaques and had fibrous tissue completely covering it on each MR image.

variations of stretch tended to appear around the fragile neovessels in carotid plaques, which may result in large deformations in this region. As a result of a local deformation, IPH may occur or extend because of rupture of neovessels. Moreover, it has been shown that surface calcification prominently elevates local stress concentrated on the plaque surface.<sup>20,21</sup> In this context, neovessels are prone to rupture where surface calcification exists. Besides IPH, surface calcification may also increase risk of neovessel rupture and thrombosis.<sup>22,23</sup> In contrast, the impact of deep

calcification on plaque maximum stress is relatively small.<sup>20,21</sup> To some degree, these studies coincide with our finding that surface calcification is a vital factor of IPH while deep calcification is not. Pathologically, neovessels sprouting from the adventitia into the plaque are associated with plaque inflammation.<sup>24,25</sup> Because deep calcifications are located near the adventitia, they may have a protective effect. Deep calcification may function as a barrier that limits the spread of inflammatory stimulus and prevents neovessels from growing into the plaque,<sup>26</sup> thereby reducing IPH.

**Table 4. Association Between Characteristics of Calcification and Presence of Intraplaque Hemorrhage**

	Univariate Regression		Model 1		Model 2	
	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value
CA	4.4 (1.6–12.5)	0.005	3.8 (1.3–11.6)	0.020	5.1 (1.8–14.5)	0.002
Single CA	1.5 (0.4–5.2)	0.532	1.2 (0.3–4.6)	0.742	1.8 (0.5–6.9)	0.360
Multiple CA	7.6 (2.6–22.1)	<0.001	7.2 (2.3–23.3)	0.001	10.1 (3.3–30.4)	<0.001
Surface CA	12.2 (2.9–50.7)	0.002	13.7 (2.6–73.5)	0.002	29.4 (4.1–210.8)	<0.001
Mixed CA	12.4 (3.6–42.5)	<0.001	13.9 (3.7–52.3)	<0.001	27.9 (7.3–107.1)	<0.001
Deep CA	1.3 (0.3–5.1)	0.657	1.1 (0.3–4.1)	0.919	1.7 (0.5–5.6)	0.414

Model 1: adjusted for age and low-density lipoprotein. Model 2: adjusted for age, low-density lipoprotein, max plaque thickness, and maximum soft plaque thickness. CA indicates calcification; CI, confidence interval; and OR, odds ratio.



We found that the presence of multiple or mixed calcifications was associated with carotid IPH. In the Rotterdam study, van den Bouwhuijsen et al<sup>13</sup> noted that calcification volume was independently associated with the prevalence of IPH within an asymptomatic population. Tissue biomechanical properties may play a role in the interaction between calcification and IPH. Calcification is much stiffer and less likely to deform compared with softer surrounding compositions such as neovessels, lipid-rich necrotic core, and IPH.<sup>27,28</sup> Regions of calcification also exhibit lower strain rates compared with regions with soft compositions. Thus, failure stress, which can result in the rupture of neovessels, may occur at the interface between calcifications and soft compositions.<sup>29</sup> Compared with single calcification, multiple calcifications may also increase the interface surface area and therefore the probability of neovessel rupture. In addition, the distribution of biomechanical stress in plaques becomes more complex and asymmetrical in plaques with multiple calcifications. Our findings on the association between quantity of calcification and IPH may lend further insight into the mechanisms of IPH pathogenesis.

### Limitations

This study has several limitations. First, our study is based on cross-sectional data. Prospective studies are warranted to investigate the role of calcification in occurrence with IPH and its expansion. Second, identification of calcification by 3-dimensional time of flight MRI or multicontrast MRI is also a limitation. This is because detection of surface calcifications may be limited by flow artifacts, which time of flight is prone to. In addition, ulcerations can create flow voids with low signal that mimics calcification. Moreover, MRI has substantial difficulty in detecting microcalcification. Previous evidence has shown that microcalcifications in carotid plaque fibrous caps are associated with plaque rupture because of increasing local stress concentrations and interfacial debonding.<sup>30–32</sup> Because the size of microcalcification is far less than the resolution of clinical MRI, quantity of calcification measured in this study may be underestimated. Future studies using more sensitive imaging sequences for detection of microcalcification, such as susceptibility weighted imaging,<sup>33</sup> are suggested. Because computed tomography angiography has significantly higher accuracy in detecting calcification, a combination of computed tomography angiography and MRI may improve accuracy in obtaining further information about calcification and IPH. Third, in this study, we did not have the chance to validate the performance of multicontrast MRI in identifying different types of calcifications with histology. The usefulness of multicontrast MRI in identification of calcification presence has been demonstrated with accuracies of 98% and specificity of 99%.<sup>34</sup> In addition, it has been shown that, in quantification of calcification, MRI demonstrates good consistency with histology ( $r=0.74$ ;  $P<0.001$ ).<sup>35</sup> Fourth, the assessment of calcification patterns and IPH by reviewers using multicontrast MRI makes blinding difficult, which may introduce bias. Calcification can be more easily seen in images that have bright background IPH on MPRAGE and time of flight, particularly for plaques with large IPH areas. However, in our study sample, the area of most IPHs was relatively small. Identification of calcification in plaques is based on the signals in calcification compared

with other constituents (eg, IPH, lipid-rich necrotic core, and fibrous tissue) and surrounding muscle, rather than comparison with the signal of IPH alone. Fifth, in our study, it is possible to lump deep calcifications together with possible adventitial calcifications (defined in the study by Eisenmenger et al<sup>11</sup>). It may be challenging for MRI to differentiate thin adventitial calcifications from chemical shift artifacts in the interface between arterial walls and surrounding fat tissues. Finally, there are only 40 IPH in our study. To eliminate the risk of overfitting, some potential confounders such as stenosis or ulceration were not considered in the multivariate analysis. Future studies with larger sample size are needed to demonstrate the association between characteristics of calcification and presence of IPH.

### Conclusion

Surface calcification and multiple calcifications in carotid atherosclerotic plaques are independently associated with the presence of IPH, suggesting that both quantity and location of calcification may play an important role in plaque vulnerability. Our findings may provide new insights into the mechanism of intraplaque hemorrhage pathogenesis.

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### Disclosures

None.

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## Highlights

- Calcification is frequently seen in carotid plaques with intraplaque hemorrhage.
- Surface calcification in carotid atherosclerotic plaques is independently associated with presence of intraplaque hemorrhage.
- Multiple calcifications in carotid atherosclerotic plaques are independently associated with presence of intraplaque hemorrhage.
- Our findings may provide novel insights for understanding the mechanism of IPH occurrence.