This copy is for personal use only. To order printed copies, contact *reprints@rsna.org*

Change in Carotid Intraplaque Hemorrhage in Community-dwelling Subjects: A Follow-up Study Using Serial MR Imaging[1]

Quirijn J. A. van den Bouwhuijsen, MD
Mariana Selwaness, MD, PhD
Hui Tang, PhD
Wiro J. Niessen, PhD
Albert Hofman, MD, PhD
Oscar H. Franco, MD, PhD
Aad van der Lugt, MD, PhD
Meike W. Vernooij, MD, PhD

Purpose: To investigate intraplaque hemorrhage (IPH) development and change over time.

Materials and Methods: Institutional review board approval and written informed consent from all participants were obtained. From a population-based study on subclinical atherosclerosis, 40 participants with IPH at baseline magnetic resonance (MR) imaging (53 carotids with IPH) were randomly selected and were matched with 27 control subjects (53 carotids without IPH) to undergo a second MR examination (mean interval, 17 months ± 4 [standard deviation]) to assess IPH change. IPH volume change was evaluated by using both a visual rating scale and an automated volumetric segmentation tool. Cardiovascular risk factors for IPH volume change were investigated with linear regression analyses.

Results: IPH remained present in 50 (94%) of the 53 carotids with IPH at baseline, and it developed in five (7%) of the 40 carotids without IPH at baseline. Visual progression of IPH volume was present in 14 (26%) of the 53 carotids with IPH at baseline, and regression was present in 16 (30%). Mean quantitative change in IPH volume was −13.7 mm^3 ± 62.6 per year of follow-up. Male sex (men vs women, 37.7 mm^3; 95% confidence interval [CI]: 11.0, 64.4; P = .006), smoking (smokers vs nonsmokers, 45.2 mm^3; 95% CI: 7.1, 83.4; P = .020), and hypertension (subjects with hypertension vs those without hypertension, 32.5 mm^3; 95% CI: 7.7, 57.2; P = .010) were associated with IPH volume change.

Conclusion: During 17 months of follow-up, both visual progression and regression of IPH volume occurs, whereas quantitatively IPH volume decreases. This suggests that IPH is a dynamic process with potential for either growth or resolution over time.

©RSNA, 2016

Online supplemental material is available for this article.

[1] From the Departments of Epidemiology (Q.J.A.v.d.B., M.S., A.H., O.H.F., M.W.V.), Radiology (Q.J.A.v.d.B., M.S., H.T., W.J.N., A.v.d.L., M.W.V.), and Medical Informatics (H.T., W.J.N.), Erasmus Medical Center, 's-Gravendijkwal 230, PO Box 2040, 3000CA Rotterdam, the Netherlands; and Department of Imaging Science and Technology, Faculty of Applied Sciences, Delft University of Technology, Delft, the Netherlands (H.T., W.J.N.). Received August 19, 2015; revision requested October 13; revision received February 8, 2016; accepted March 7; final version accepted June 9. **Address correspondence to** A.v.d.L. (e-mail: *a.vanderlugt@erasmusmc.nl*).

Supported by Netherlands Heart Foundation (2006B206, 2009B044) and Netherlands Organization for Scientific Research (Vici, grant No. 918-76-619).

Q.J.A.v.d.B. and M.S. contributed equally to this work.

©RSNA, 2016

Vulnerable atherosclerotic plaques are considered to lead to cardiovascular events through plaque rupture and thromboembolism (1). Magnetic resonance (MR) imaging is a validated tool with which to assess imaging morphology and composition of the carotid atherosclerotic plaque. Intraplaque hemorrhage (IPH) has been shown to be an important marker of plaque progression and destabilization through proinflammatory response, lipid accumulation, and accelerated progression in plaque burden (2–4).

It is important to understand the development and natural course of IPH to identify potentially modifiable factors; however, these processes remain poorly understood. In a previous hospital-based serial MR imaging study, IPH was related to an increase in necrotic core size and plaque burden both in subjects with advanced carotid atherosclerotic disease (range, 50%–79% stenosis) and in subjects with less than 50% stenosis. In previous studies, changes in IPH presence over time were shown in only those subjects with advanced carotid stenosis (5–7). A carotid atherosclerosis score that associates with IPH presence has been described (8); however, in a longitudinal study, this score could not be used to predict development of new IPH (9). In all these previous studies, researchers considered IPH as only a dichotomous variable and did not study change in IPH volume over time. One report used IPH area size to predict cerebrovascular risk; however, MR imaging was performed only at baseline (10).

Quantification of change in IPH volume in asymptomatic individuals may contribute to identification of new risk factors for IPH development and progression and may yield further clues for effective cardiovascular prevention.

We used repeated MR imaging measurements to evaluate IPH development and change in IPH volume over 1.5 years of follow-up in asymptomatic subjects and to identify risk factors for IPH progression. We used a nested case-control design in 40 subjects with IPH at baseline and 27 subjects without IPH at baseline from a community-dwelling population of subjects older than 45 years.

Advances in Knowledge

- By using serial MR imaging in the detection and follow up of carotid intraplaque hemorrhage (IPH), we showed that qualitatively both progression (26%) and regression (30%) in IPH volume occurred frequently during follow-up, whereas quantitatively overall IPH volume decreased by a mean of −13.7 mm^3 ± 62.6 per year.
- We found smoking and hypertension to be the most important potentially modifiable risk factors for increase in IPH volume; in smokers versus nonsmokers, the mean increase in IPH over 1.4 years was 45.2 mm^3 (95% confidence interval [CI]: 7.1, 83.4; P = .020), and in subjects with hypertension versus those without hypertension, mean increase in IPH over 1.4 years was 32.5 mm^3 (95% CI: 7.7, 57.2; P = .010).

Materials and Methods

Study Population

This study is embedded in the Rotterdam study (11), a prospective population-based cohort study limited to persons 45 years old or older. At study entry and every 3–4 years thereafter, all Rotterdam study participants are re-examined at a dedicated research center. From October 2007 on, carotid MR imaging was incorporated in the Rotterdam study in all persons with carotid intima-media thickening in one or both carotid arteries on sonograms (12). Between October 2007 and November 2008, 360 participants underwent a complete MR imaging examination and image review according to previously described protocols (12). In 113 of these 360 participants, there was uni- or bilateral carotid IPH. For this case-control study, we randomly invited 40 subjects with IPH and selected 30 age-, sex-, and plaque thickness–matched subjects without IPH (n = 30) to undergo serial MR imaging after approximately 1.5 years. Subjects with prior carotid endarterectomy were not invited. Three invited control subjects refused to participate. All 40 invited subjects and the remaining 27 control subjects underwent serial imaging (67 participants in total). The mean interval between examinations was 17 months ± 4 (standard deviation) (range, 11–24 months). The Rotterdam study has been approved by the institutional review board of Erasmus Medical Center (Rotterdam, the Netherlands) according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants.

Implication for Patient Care

- A better understanding of factors driving progression of vulnerable plaque components might ultimately lead to early treatment and prevention of IPH.

MR Image Acquisition and Review

All MR images were obtained with the same 1.5-T unit (GE Signa Excite II; GE Healthcare, Milwaukee, Wis) at both time points with a bilateral phased-array surface coil. The MR imaging protocol has been described in detail

Published online before print
10.1148/radiol.2016151806 **Content code:** NR

Radiology 2017; 282:526–533

Abbreviations:
CI = confidence interval
IPH = intraplaque hemorrhage

Author contributions:
Guarantors of integrity of entire study, Q.J.A.v.d.B., A.v.d.L., M.W.V.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; agrees to ensure any questions related to the work are appropriately resolved, all authors; literature research, Q.J.A.v.d.B., H.T., A.v.d.L.; clinical studies, Q.J.A.v.d.B., M.S., H.T., A.v.d.L.; statistical analysis, Q.J.A.v.d.B., M.S., H.T.; and manuscript editing, all authors

Conflicts of interest are listed at the end of this article.

elsewhere (12) and included an intermediate-weighted fast spin-echo black-blood sequence and a three-dimensional T1-weighted gradient-echo sequence. Carotid wall thickness (in millimeters) and stenosis (in percentage) based on the North American Symptomatic Carotid Endarterectomy Trial (or NASCET) criteria (13) were manually assessed on MR images obtained with the intermediate-weighted fast spin-echo black-blood sequence. Evaluation of the presence of IPH was performed by one trained observer (Q.J.A.v.d.B., 4 years of experience) using the three-dimensional T1-weighted gradient-echo MR imaging sequence; presence of IPH was defined as a hyperintense region in the atherosclerotic plaque. Previously assessed interobserver reliability between this reader (Q.J.A.v.d.B.) and a second reader (A.v.d.L., 8 years of experience) was very good, with Cohen κ values of 0.85–0.95 (12).

Qualitative and Quantitative Assessment of Change in IPH

Qualitative assessment of IPH change from baseline to follow-up was performed by two independent raters (Q.J.A.v.d.B. and M.S., 3 years of experience) in consensus using a visual rating scale in all 67 participants. Both raters were trained in detection of IPH and were working under the supervision of a neuroradiologist (A.v.d.L.). The observers were presented with the baseline and follow-up T1-weighted MR images in a random order and were blinded to information about when the images were obtained. First, the presence of IPH was scored, and subsequently a change in IPH size was scored on a five-point scale, ranging from −2 (remarkable regression of more than 50% or disappearance of IPH) to +2 (remarkable progression of more than 50% or newly developed IPH). If there was no apparent difference, it was scored as 0. Inter- and intraobserver reliability (Q.J.A.v.d.B.. M.S.) with the visual rating scale (n = 20) was good, with Cohen κ values of 0.81 and 0.89, respectively.

For quantitative analysis of IPH volume change, we used a semiautomated algorithm developed in-house to measure IPH volume (in cubic millimeters) at baseline and at follow-up (Fig E1 [online]) (14). In short, IPH segmentation was performed with a piecewise smooth regional level set method, which was initialized by three user-selected seed points. A region of interest was then automatically generated around the seed points. The accuracy, reproducibility, and robustness of this semiautomated method have been described previously, with a dice similarity coefficient of 0.88 when compared with manual segmentation and an interscan robustness correlation dice similarity coefficient of 0.99 (14). For 20 examinations performed less than 1 week apart in a reproducibility assessment, pooled standard deviation for this quantitative IPH measurement was 9.5 mm^3 at a mean IPH volume of 143.5 mm^3, with a coefficient of variation of 7.6%.

Cardiovascular Risk Factors

Covariates were ascertained at the time of baseline MR imaging or at the time of closest previous study center visit and home interview by using standard procedures, as described previously (15). The body mass index was calculated based on weight and height. Blood pressure was measured at the study center visit. Serum total cholesterol and high-density lipoprotein cholesterol levels were measured. Smoking status was classified as current, past, or never. Diabetes mellitus was considered present when the fasting blood glucose level exceeded 7.0 mmol/L, when the nonfasting glucose level exceeded 11.0 mmol/L, or when antidiabetic medication was used. Hypertension was considered present if the subject used antihypertensive medication or if his or her blood pressure was 140/90 mmHg or higher. History of myocardial infarction or stroke was assessed until the date of inclusion. All participants were observed for occurrence of major coronary or cerebrovascular events during follow-up (16).

Data Analysis

Maximum plaque thickness (in millimeters), degree of carotid stenosis, presence of IPH, and IPH volume (in cubic millimeters) were measured on both sides in each carotid artery at baseline and at follow-up, and changes were evaluated per carotid artery and were compared between carotids with IPH and those without IPH at baseline. Differences between subjects with IPH and control subjects or between baseline and follow-up were calculated with the Student t test or paired t test for continuous variables and with the χ^2 test or McNemar test for categorical variables, respectively. Correlation between IPH volume change assessed with the automated segmentation tool and with the visual rating score was studied by using Kendall τ b bivariate correlation.

We used linear regression models to analyze determinants of change in IPH volume restricted to all carotid arteries with IPH at baseline or at follow-up (17). IPH volume at follow-up (automated quantification) was chosen as an outcome in these analyses, and we adjusted for IPH volume at baseline (univariate model). Additional adjustments were performed (multivariate model) for age, sex, carotid plaque thickness, time between examinations, and cardiovascular risk factors (smoking, hypertension, hypercholesterolemia, and diabetes mellitus). Additionally, we used ordinal logistic regression to analyze determinants of change in visual rating score of IPH volume restricted to all carotid arteries with IPH at baseline or follow-up (Appendix E1 [online]).

To adjust for the correlation and potential confounding bias between both carotid arteries within one participant, we followed a generalized estimation equation approach, with an independent or unstructured working correlation matrix that included two levels per participant, namely the left and right carotid artery. The generalized estimation equation can handle correlated response data, particularly if responses are binary, preventing possible bias from paired arteries not being independent data points. Statistical software (SPSS, version 21.0; IBM, Armonk, NY) was used for all analyses. The P value threshold for significance was .05.

Table 1

Subject Characteristics at Baseline

Characteristic	All Subjects ($n = 67$)	Case Subjects ($n = 40$)	Control Subjects ($n = 27$)	*P* Value
Age (y)*	71.3 ± 10.9	71.0 ± 11.2	71.9 ± 10.7	.39
Male sex	48 (72)	30 (75)	18 (67)	.46
Hypertension	47 (70)	28 (70)	19 (70)	.98
Diastolic blood pressure (mmHg)*	80 ± 13	78 ± 13	82 ± 12	.04
Systolic blood pressure (mmHg)*	146 ± 22	144 ± 23	147 ± 21	.90
Use of blood pressure–lowering drugs	36 (54)	25 (62)	11 (41)	.08
Hypercholesterolemia	18 (27)	13 (32)	5 (19)	.21
High-density lipoprotein level (mmol/L)*	1.3 ± 0.4	1.2 ± 0.3	1.5 ± 0.4	.02
Use of cholesterol-lowering medication	29 (43)	17 (42)	12 (44)	.88
Body mass index (kg/m^2)*	27.4 ± 3.8	27.6 ± 3.9	27.1 ± 3.8	.86
Diabetes mellitus	16 (24)	12 (30)	4 (15)	.15
Current or past smoking	53 (79)	35 (88)	18 (67)	.04
Previous cardiovascular disease	10 (15)	5 (12.	5 (19)	.50
Previous stroke	4 (6)	4 (10)	0 (0)	.09
Maximum plaque thickness (mm)*	4.2 ± 1.2	3.9 ± 0.8	4.5 ± 1.3	.01
Interval between two MR imaging examinations (y)*	1.3 ± 0.3	1.3 ± 0.3	1.4 ± 0.3	.03

Note.—Unless otherwise indicated, data are number of subjects, and data in parentheses are percentages. *P* values for difference between subjects with IPH and control subjects were calculated with the Student *t* test for continuous variables and with the χ^2 test for categorical variables.

* Data are mean ± standard deviation.

Results

Table 1 shows characteristics of all 67 subjects in the study population. Figure 1 is a flowchart showing breakdown of all 124 carotid arteries included in the study. In the 40 participants with IPH at baseline MR imaging, bilateral carotid wall thickening was present in 34; in the remaining six, carotid wall thickening was unilateral (in total, there were 74 carotid arteries with plaques). In 53 (72%) of these carotid plaques, IPH was present at baseline. In the 27 matched control subjects without IPH, bilateral carotid wall thickening was present in 23, unilateral wall thickening was present in three, and carotid artery occlusion was present in one (in total, there were 50 carotid arteries with plaque, all without IPH) (Fig 1).

Visual Rating

Table 2 shows carotid plaque characteristics at baseline and at follow-up. New IPH developed in five (7%) of 71 carotid arteries without IPH at baseline. IPH volume progressed remarkably in three (6%) and moderately in 11 (21%) of the 53 carotid arteries with IPH at baseline; there was no change in 23 (43%) arteries, and IPH volume regressed moderately in 12 (23%) and remarkably in four (8%) arteries (Fig 2). Table E1 (online) shows cardiovascular risk factors associated with change in visual rating score of IPH volume. Hypertension ($P = .070$, not significant) and current smoking ($P = .020$) were associated with an increase in the visual rating score.

Automated Rating

Automated volumetric segmentation in the five carotid arteries with newly developed IPH showed an IPH volume of 59.2 mm^3 ± 34.7 during 17 months of follow-up, corresponding to an annual IPH volume growth of 44.7 mm^3 ± 28.3. Automated quantification of all arteries with IPH at baseline showed a mean decrease in IPH volume of −19.8 mm^3 ± 79.9 during follow-up, corresponding to an annual change in IPH volume of −13.7 mm^3 ± 62.6. In 11 (21%) carotid arteries with IPH, there was an increase in IPH volume of more than 25 mm^3 (mean increase, 70 mm^3 ± 49). Conversely, in 16 (30%) carotid arteries with IPH, there was a decrease in IPH volume of more than 25 mm^3 (mean decrease, 82 mm^3 ± 67). Correlation between automated IPH volume change and the visual rating scale was 0.41 ($P < .001$). Carotid arteries with persistent IPH at baseline and at follow-up, those with disappearance of IPH at follow-up, those with new IPH at follow-up, and those without IPH at either time point showed a mean annual change in maximum plaque thickness of 0.2 mm ± 0.6, −0.1 mm ± 0.1, 0.2 mm ± 0.6, and 0.1 mm ± 0.4, respectively ($P > .05$).

Table 3 shows cardiovascular risk factors associated with change in carotid IPH volume. Male sex was associated with IPH volume progression (mean annual increase in IPH volume in male participants vs female participants, 37.7 mm^3; 95% confidence interval [CI]: 11.1, 64.4; $P = .006$). This association was independent from age and other factors (multivariate model) ($P = .002$). Furthermore, hypertension (mean increase in IPH volume in participants with hypertension vs those without hypertension, 32.5 mm^3; 95% CI: 7.7, 57.2; $P = .010$) and smoking (mean increase in IPH volume in current smokers vs those who never smoked, 45.2 mm^3; 95% CI: 7.1, 83.4; $P = .020$) were associated with progression of IPH volume. These associations were borderline significant ($P = .060$ and $P = .057$, respectively) when adjusted for age and other factors (multivariate model). Associations were unaffected when we restricted the analysis in the subgroup of carotid plaques with IPH at baseline ($n = 53$), thereby eliminating the effect of increasing plaque thickness and degree of stenosis in plaques with new IPH (Table E1 [online]). In the subgroup of five subjects with new IPH, three were male, all five had hypertension, and two were current smokers.

Conversely, albeit not significant, for carotid stenosis of more than 50%, there was the suggestion of a relationship with regression of IPH volume (−32.6 mm^3; 95% CI: −84.5, 19.2;

Figure 1

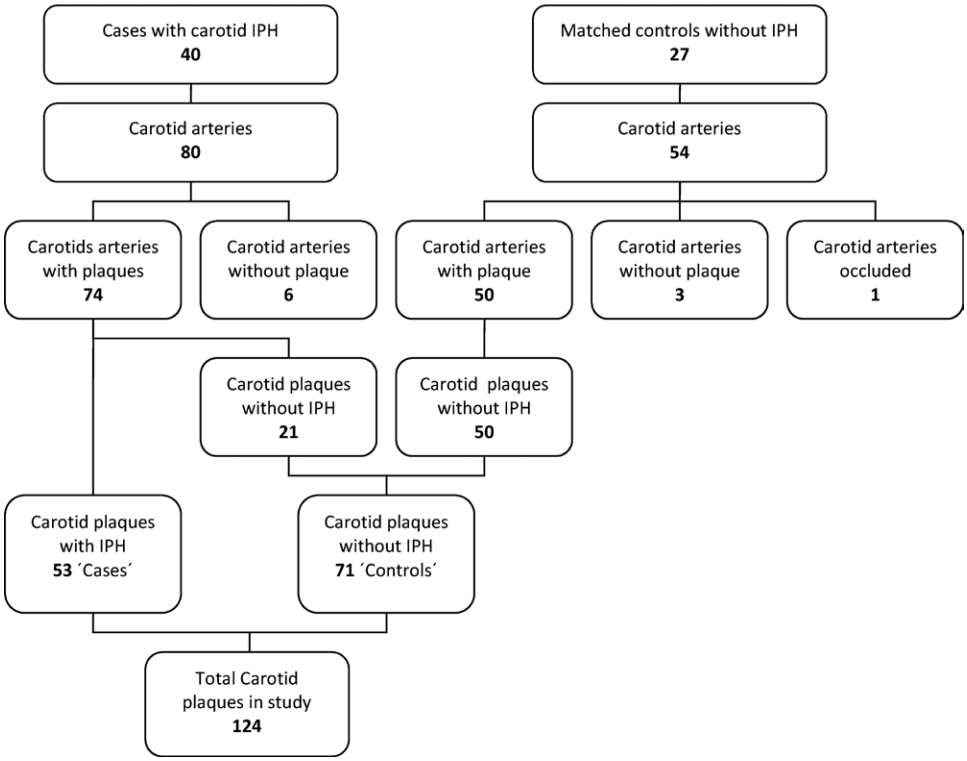

Figure 1: Study population flowchart shows number of carotid arteries with and without IPH at baseline. A carotid plaque was considered a carotid wall thickness of more than 2 mm on intermediate-weighted fast spin-echo black-blood MR images.

Table 2

Baseline and Follow-up Measurements of Plaque Characteristics in 124 Carotid Plaques

	Carotids with IPH at Baseline (n = 53)			Carotids without IPH at Baseline (n = 71)		
Characteristic	Baseline	Follow-up	*P* Value	Baseline	Follow-up	*P* Value
Visual presence of IPH	53 (100)	50 (94)	.72	0 (0)	5 (7)	.56
Visual change at follow-up						
Remarkable progression	…	3 (6)	…	…	5 (7)	…
Moderate progression	…	11 (21)	…	…	…	…
No change	…	23 (43)	…	…	…	…
Moderate regression	…	12 (23)	…	…	…	…
Remarkable regression	…	4 (8)	…	…	…	…
Volume (mm^3)*	109.3 ± 120.0	89.9 ± 103.6	.08	…	59.2 ± 34.7	…
Maximum plaque thickness (mm)*	4.1 ± 1.1	4.3 ± 1.1	.07	3.4 ± 0.9	3.6 ± 0.8	.06
Degree of stenosis (%)*	23.4 ± 19.0	24.9 ± 19.8	.35	10.7 ± 13.9	13.3 ± 15.6	.04

Note.—Unless otherwise indicated, data are number of subjects, and data in parentheses are percentages. *P* values for difference between baseline and follow-up values were evaluated with a paired *t* test for continuous variables and with a McNemar test for categorical variables.

* Data are mean ± standard deviation.

P = .217), and this association became stronger when additionally adjusted for carotid wall thickness (−36.5 mm^3; 95% CI: −77.5, 4.4; P = .081).

Overall, 43% of the subjects used cholesterol-lowering medication. This percentage was comparable in all groups with visual IPH change. Use of cholesterol-lowering medication also was not associated with change in absolute IPH volume (P = .31).

Figure 2

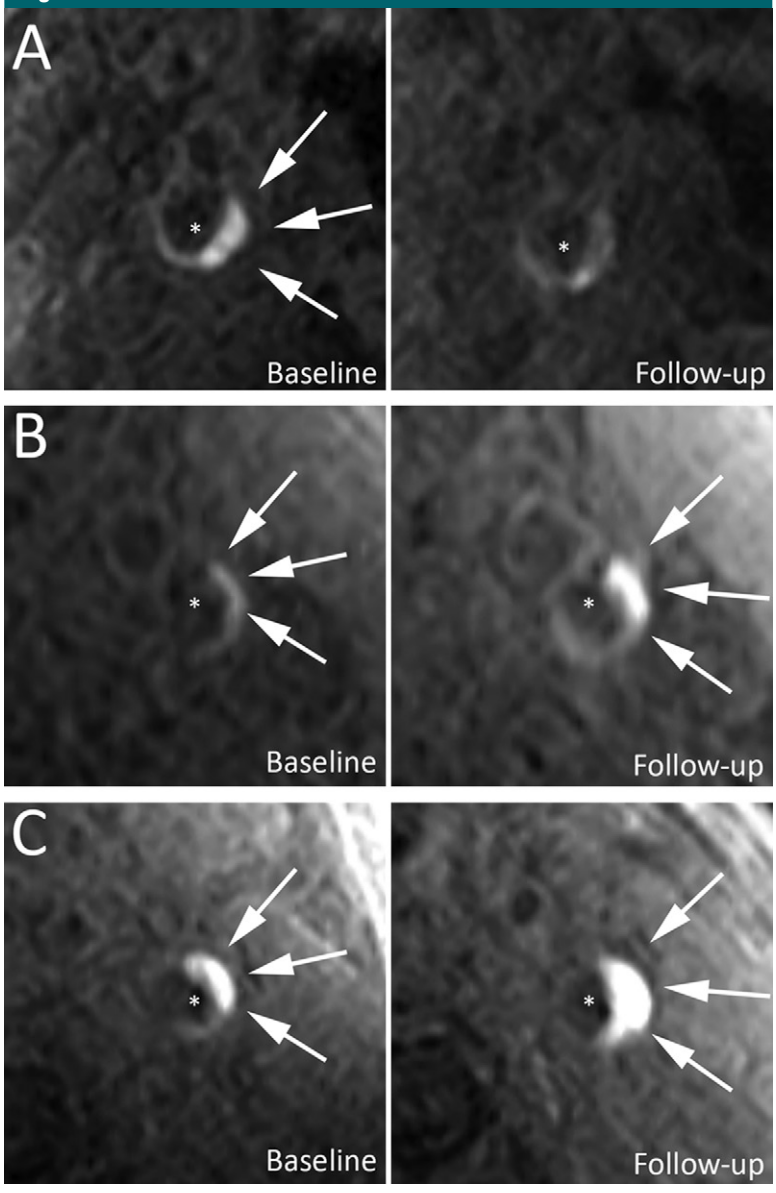

Figure 2: Examples of remarkable regression and progression of IPH volume during follow-up. T1-weighted gradient-echo MR images of carotid atherosclerotic plaques with IPH in three subjects at baseline and follow-up. *A*, Images in a 63-year-old man at baseline and at 1-year follow-up. With the visual rating scale, there is a remarkable decrease in IPH volume. IPH volume regression was −255 mm^3, as measured with the semiautomated tool. *B*, Images in a 60-year-old man at baseline and at 1-year follow-up. With the visual rating scale, there is a moderate increase in IPH volume. IPH volume progression was 62 mm^3, as measured with the semiautomated tool. *C*, Images in a 56-year-old man at baseline and at 1.5-year follow-up. With the visual rating scale, there is a remarkable increase in IPH volume. IPH volume progression was 173 mm^3, as measured with the semiautomated tool. Note the lumen of the internal carotid artery (∗) and the IPH (arrows) (the area of high signal intensity) on all images.

There were no cardiovascular or cerebrovascular events during follow-up in any of the 67 subjects.

Discussion

In this case-control MR imaging study in community-dwelling subjects with carotid atherosclerosis, we showed that qualitatively, both progression and regression in IPH volume occur frequently over time, whereas quantitatively overall IPH volume decreases, albeit with wide variation. Male sex, smoking, and hypertension were all associated with an increase in IPH volume over time. Conversely, moderate to severe carotid stenosis seemed to be associated with a decrease in IPH volume.

In contrast to findings in previous studies (5–7), subjects in our study were not symptomatic but were randomly selected from a large sample of subjects with preclinical atherosclerosis. Thus, our results are more representative of the natural course of IPH and are of interest in view of potential preventive measures.

MR imaging signal for IPH remained detectable in the majority of carotid plaques after 17 months. Our findings are in agreement with those of Yamada et al (18), who reported that in one of 30 carotid arteries, MR imaging signal intensity for IPH changed during a median interval of approximately 9 months. Also, Takaya et al (6) reported that IPH remained detectable in the same plaque after 18 months. It was suggested that either *(a)* IPH does not resolve by an atypical degradation of hemoglobin or *(b)* IPH recurs in the same plaque repetitively (19). In our present study, we expounded on these findings by showing that despite persistence of high signal intensity within the plaques, both visual rating and automated assessment indicate that IPH may regress over a short follow-up period.

It is important to assess potential modifiable risk factors for development or growth of IPH, as IPH is considered a major risk indicator for plaque instability and subsequent cerebrovascular events (2–4,20). In a previous study in 1006 asymptomatic

Table 3

Cardiovascular Risk Factors and Carotid IPH Volume Change at Follow-up

Characteristic	Model 1 (mm^3)	*P* Value	Model 2 (mm^3)	*P* Value
Follow-up time	−2.4 (−51.3; 46.5)	.924	−6.2 (−67.2; 54.8)	.843
Male sex	37.7 (11.1; 64.4)	.006	40.5 (14.5; 66.5)	.002
Age	−0.4 (−2.1; 1.3)	.644	0.5 (−1.1; 2.2)	.512
Maximum plaque thickness	5.0 (−10.5; 20.5)	.527	7.4 (−9.3; 24.0)	.385
Degree of carotid stenosis	0.0 (−1.0; 1.0)	.972	0.1 (−1.1; 1.4)	.837
Carotid stenosis of ≥50%	−32.6 (−84.5; 19.2)	.217	−26.8 (−78.4; 24.8)	.308
Smoking				
Past vs never	34.3 (10.0; 58.5)	.006	12.0 (−12.6–36.6)	.340
Current vs never	45.2 (7.1; 83.4)	.020	35.0 (−1.0; 71.1)	.057
Body mass index	−1.4 (−5.4; 2.5)	.486	−1.3 (−5.2; 2.7)	.539
Hypertension	32.5 (7.7; 57.2)	.010	24.4 (−1.0; 49.8)	.060
Diabetes mellitus	−4.3 (−40.6; 31.9)	.815	−5.0 (−43.5; 33.6)	.798
Hypercholesterolemia	−18.6 (−55.3; 18.1)	.320	−14.7 (−60.1; 30.7)	.525
High-density lipoprotein level	−33.6 (−93.5; 26.2)	.271	−24.9 (−83.9; 34.1)	.408
Use of cholesterol-lowering medication	20.1 (−18.4; 58.8)	.306	26.2 (−12.5; 64.9)	.184

Note.—Data are difference in IPH volume per unit increase of the cardiovascular risk factors at baseline. Data in parentheses are the 95% CI. Model 1= univariate analysis. Model 2 = multivariate analysis adjusted for baseline IPH volume, age, sex, and all other covariates in the univariate analysis with $P \leq .10$ (smoking and hypertension), when applicable.

community-dwelling subjects with atherosclerotic carotid plaques, male sex, hypertension, and current smoking were major risk factors for presence of IPH (12). In the current study, we extended these findings to IPH volumetric change. We showed male sex, hypertension, and smoking were independently associated with IPH growth. Nicotine has been found to play an important stimulating role in angiogenesis and plaque growth, which may explain the growth in IPH volume that we found in smokers (21,22). In vitro studies that subject the vessel wall in specific vascular smooth muscle cells to cyclic strain showed upregulation of vascular endothelial growth factor, which is an indicator of angiogenesis (23). This may explain our findings in subjects with hypertension. However, further investigations are necessary to elucidate the exact pathophysiologic mechanism of IPH development and growth.

In a study in 73 subjects with asymptomatic carotid stenosis of 50%–70%, a scoring system using plaque characteristics (wall thickness and lipid core) was associated with presence of IPH (8), albeit not with newly developed IPH (9). In both articles, IPH volume or growth was not quantified. Two previous serial MR imaging studies in a hospital setting showed an increase in total plaque burden in the presence of IPH (5–7), suggesting that IPH may drive plaque growth. We used plaque thickness as a proxy for plaque burden and found an equal increase in plaque thickness over time between arteries with IPH and those without, though plaques with IPH were thicker, on average. Both of these previous studies considered IPH only as a dichotomous variable. Thus, our study adds more understanding on IPH change over time.

Limitations of our study must be discussed. We have not taken the location of the IPH within the carotid plaque into account. Thus, we cannot be sure whether the IPHs observed at follow-up were at the same location as those observed at baseline or if some of these may also represent newly developed IPH. In our visual assessment, however, we found that in 94% of the subjects, IPH remained present, which is in agreement with findings in two previous studies (6,24). Still, we may have underestimated the rate of new IPH development, as this would have been scored as growth of IPH with both visual assessment and automated assessment. Our automated tool was not designed to measure vulnerable plaque components other than IPH (eg, fibrous cap thickness, lipid core size), thereby leaving out other important factors of plaque instability. Finally, we studied cardiovascular risk factors in a dichotomous distribution. Although this facilitates clinical interpretation and comparison to other studies, it may have led to a lower sensitivity in the detection of associations.

Recently, different stages of IPH have been characterized (recent, organized, amorphous). These different stages may represent the morphologic substrates of different types of repair response to the plaque hemorrhage (25). It has been reported that IPH can induce a healing response at the site of the hemorrhage with the formation of fibrous and calcified tissue, which ultimately leads to plaque stabilization (26). This could explain the decrease in mean IPH volumes with a concurrent increase in atherosclerotic plaque burden in our study. Unfortunately, we were not able to distinguish these different stages of IPH with our MR imaging protocol. The only determinant we found in our study to potentially relate to IPH regression was carotid stenosis of more than 50%. This may be explained by altered hemodynamic factors and wall shear stress in subjects with high-grade stenosis, reducing the strain on the plaque. Future studies should focus on the exact mechanism, as well as on finding other potentially modifiable determinants for regression of IPH burden, as this may affect interventional strategies to reduce plaque vulnerability.

In conclusion, serial MR imaging of carotid arteries in community-dwelling subjects with subclinical carotid atherosclerosis over a 17-month period showed new IPH development in plaques that were previously negative for IPH. In plaques containing IPH at baseline, both visual and quantitative progression and regression in IPH volume occurred, suggesting that IPH is a dynamic process with both growth and resolution over time. We found

smoking and hypertension to be the most important potentially modifiable risk factors for increase in IPH volume. Further studies are needed to corroborate our findings and to increase our understanding of the mechanisms leading to progression of atherosclerotic plaques and cerebrovascular events.

Disclosures of Conflicts of Interest: Q.J.A.v.d.B. disclosed no relevant relationships. **M.S.** disclosed no relevant relationships. **H.T.** disclosed no relevant relationships. **W.J.N.** Activities related to the present article: disclosed no relevant relationships. Activities not related to the present article: is a cofounder, shareholder, and chief scientific officer of Quantib BV. Other relationships: disclosed no relevant relationships. **A.H.** disclosed no relevant relationships. **O.H.F.** Activities related to the present article: disclosed no relevant relationships. Activities not related to the present article: received grants from Metagenics and Nestle. Other relationships: disclosed no relevant relationships. **A.v.d.L.** disclosed no relevant relationships. **M.W.V.** disclosed no relevant relationships.

References

1. Carr S, Farb A, Pearce WH, Virmani R, Yao JS. Atherosclerotic plaque rupture in symptomatic carotid artery stenosis. J Vasc Surg 1996;23(5):755–765; discussion 765–766.
2. Kolodgie FD, Gold HK, Burke AP, et al. Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med 2003;349(24):2316–2325.
3. Underhill HR, Yuan C, Yarnykh VL, et al. Predictors of surface disruption with MR imaging in asymptomatic carotid artery stenosis. AJNR Am J Neuroradiol 2010;31(3):487–493.
4. Michel JB, Virmani R, Arbustini E, Pasterkamp G. Intraplaque haemorrhages as the trigger of plaque vulnerability. Eur Heart J 2011;32(16):1977–1985, 1985a, 1985b, 1985c.
5. Sun J, Underhill HR, Hippe DS, Xue Y, Yuan C, Hatsukami TS. Sustained acceleration in carotid atherosclerotic plaque progression with intraplaque hemorrhage: a long-term time course study. JACC Cardiovasc Imaging 2012;5(8):798–804.
6. Takaya N, Yuan C, Chu B, et al. Presence of intraplaque hemorrhage stimulates progression of carotid atherosclerotic plaques: a high-resolution magnetic resonance imaging study. Circulation 2005;111(21):2768–2775.
7. Underhill HR, Yuan C, Yarnykh VL, et al. Arterial remodeling in [corrected] subclinical carotid artery disease. JACC Cardiovasc Imaging 2009;2(12):1381–1389.
8. Underhill HR, Hatsukami TS, Cai J, et al. A noninvasive imaging approach to assess plaque severity: the carotid atherosclerosis score. AJNR Am J Neuroradiol 2010;31(6):1068–1075.
9. Xu D, Hippe DS, Underhill HR, et al. Prediction of high-risk plaque development and plaque progression with the carotid atherosclerosis score. JACC Cardiovasc Imaging 2014;7(4):366–373.
10. Takaya N, Yuan C, Chu B, et al. Association between carotid plaque characteristics and subsequent ischemic cerebrovascular events: a prospective assessment with MRI: initial results. Stroke 2006;37(3):818–823.
11. Hofman A, Darwish Murad S, van Duijn CM, et al. The Rotterdam Study: 2014 objectives and design update. Eur J Epidemiol 2013;28(11):889–926.
12. van den Bouwhuijsen QJ, Vernooij MW, Hofman A, Krestin GP, van der Lugt A, Witteman JC. Determinants of magnetic resonance imaging detected carotid plaque components: the Rotterdam Study. Eur Heart J 2012;33(2):221–229.
13. Barnett HJ, Taylor DW, Eliasziw M, et al. Benefit of carotid endarterectomy in patients with symptomatic moderate or severe stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators. N Engl J Med 1998;339(20):1415–1425.
14. Tang H, Selwaness M, Hameeteman R, et al. Semi-automatic MRI segmentation and volume quantification of intra-plaque hemorrhage. Int J CARS 2015;10(1):67–74.
15. Vliegenthart R, Oudkerk M, Hofman A, et al. Coronary calcification improves cardiovascular risk prediction in the elderly. Circulation 2005;112(4):572–577.
16. Bos D, Portegies ML, van der Lugt A, et al. Intracranial carotid artery atherosclerosis and the risk of stroke in whites: the Rotterdam Study. JAMA Neurol 2014;71(4):405–411.
17. Vickers AJ, Altman DG. Statistics notes: analysing controlled trials with baseline and follow up measurements. BMJ 2001;323(7321):1123–1124.
18. Yamada N, Higashi M, Otsubo R, et al. Association between signal hyperintensity on T1-weighted MR imaging of carotid plaques and ipsilateral ischemic events. AJNR Am J Neuroradiol 2007;28(2):287–292.
19. Virmani R, Kolodgie FD, Burke AP, et al. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. Arterioscler Thromb Vasc Biol 2005;25(10):2054–2061.
20. Altaf N, Daniels L, Morgan PS, et al. Detection of intraplaque hemorrhage by magnetic resonance imaging in symptomatic patients with mild to moderate carotid stenosis predicts recurrent neurological events. J Vasc Surg 2008;47(2):337–342.
21. Heeschen C, Jang JJ, Weis M, et al. Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. Nat Med 2001;7(7):833–839.
22. Cooke JP. Angiogenesis and the role of the endothelial nicotinic acetylcholine receptor. Life Sci 2007;80(24-25):2347–2351.
23. Schad JF, Meltzer KR, Hicks MR, Beutler DS, Cao TV, Standley PR. Cyclic strain upregulates VEGF and attenuates proliferation of vascular smooth muscle cells. Vasc Cell 2011;3:21.
24. Simpson RJ, Akwei S, Hosseini AA, MacSweeney ST, Auer DP, Altaf N. MR imaging-detected carotid plaque hemorrhage is stable for 2 years and a marker for stenosis progression. AJNR Am J Neuroradiol 2015;36(6):1171–1175.
25. Derksen WJ, Peeters W, van Lammeren GW, et al. Different stages of intraplaque hemorrhage are associated with different plaque phenotypes: a large histopathological study in 794 carotid and 276 femoral endarterectomy specimens. Atherosclerosis 2011;218(2):369–377.
26. Moreno PR, Purushothaman KR, Sirol M, Levy AP, Fuster V. Neovascularization in human atherosclerosis. Circulation 2006;113(18):2245–2252.