Time-Dependent Changes in Atherosclerotic Plaque Composition in Patients Undergoing Carotid Surgery

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Background—Time-dependent trends in the incidence of cardiovascular disease have been reported in high-income countries. Because atherosclerosis underlies the majority of cardiovascular diseases, we investigated temporal changes in the composition of atherosclerotic plaques removed from patients undergoing carotid endarterectomy.

Methods and Results—The Athero-Express study is an ongoing, longitudinal, vascular biobank study that includes the collection of atherosclerotic plaques of patients undergoing primary carotid endarterectomy in the province of Utrecht from 2002 to 2011. Histopathologic features of plaques of 1583 patients were analyzed in intervals of 2 years. The analysis included quantification of collagen, calcifications, lipid cores, plaque thrombosis, macrophages, smooth muscle cells, and microvessels. Large atheroma, plaque thrombosis, macrophages, and calcifications were less frequently observed over time, with adjusted odds ratios of 0.72 (95% confidence interval, 0.650-0.789), 0.62 (95% confidence interval, 0.569-0.679), 0.87 (95% confidence interval, 0.800-0.940), and 0.75 (95% confidence interval, 0.692-0.816) per 2-year increase in time, respectively. These changes in plaque characteristics were consistently observed in patient subgroups presenting with stroke, transient ischemic attack, ocular symptoms, and asymptomatic patients. Concomitantly, risk factor management and secondary prevention strategies among vascular patients scheduled for carotid endarterectomy significantly improved over the past decade.

Conclusions—In conclusion, over the past decade, atherosclerotic plaques harvested during carotid endarterectomy show a time-dependent change in plaque composition characterized by a decrease in features currently believed to be causal for plaque instability. This appears to go hand in hand with improvements in risk factor management. (Circulation. 2014;129:2269-2276.)

Key Words: endarterectomy, carotid histology plaque, atherosclerotic primary prevention

Population-based studies demonstrate an age-adjusted decrease in manifestations of atherosclerotic cardiovascular diseases, such as stroke and coronary heart disease, over the last decades in high-income countries. The composition of atherosclerotic plaques is considered to reflect the local atherosclerotic disease severity. Indeed, autopsy and clinical pathology studies have revealed that thin-capped atheromatous plaques are associated with rupture resulting in thrombotic occlusion and a subsequent acute cardiovascular event. In addition, a minority of thrombotic events are caused by plaque erosion in the presence of a fibrous lesion.

Clinical Perspective on p 2276

Autopsy studies have provided many insights into the atherosclerotic plaque characteristics that are associated with cardiovascular events. Yet, there is no report on temporal changes in plaque phenotypes during the last decades coinciding with the decreased incidence of cardiovascular disease reported in high-income countries. To investigate time-dependent changes in atherosclerotic plaque characteristics, we used the Athero-Express study and analyzed histologic features of >1500 plaques removed during carotid endarterectomy from patients with similar symptomatology included from 2002 to 2011.

Methods

Athero-Express

Study Population

Athero-Express is an ongoing, longitudinal, biobank study collecting carotid atherosclerotic plaques from patients who underwent carotid endarterectomy in the University Medical Center Utrecht.
or the St Antonius Hospital Nieuwegein. Medical ethics commit-
tees of both participating centers approved the study, and all of the
included patients provided written informed consent. All of the
patients who underwent carotid endarterectomy from the start of
the Athero-Express in 2002 up to 2011 were included. Patients
were excluded from the study if informed consent was lacking. Indica-
tion for surgery was based on international guidelines for symptomatic
and asymptomatic carotid stenosis. All indications were reviewed
by a multidisciplinary team of vascular specialists before surgery.
Patient data were obtained via standardized questionnaires and pre-
operative admission charts.

Plaque Processing and Assessment
Atherosclerotic plaques of patients were harvested during carotid end-
arterectomy according to a standardized and previously reported pro-
tocol. Briefly, carotid plaques were divided into segments of 5-mm
thickness. The section with the largest plaque burden was classified
as a culprit lesion and subjected to immunohistochemical staining.
Plaques were stained for macrophages (CD68), smooth muscle cells
(α-smooth, collagen (Picro-Sirius Red), microvessels (CD34), and
presence of plaque thrombosis (consisting of the combination of luminal
thrombi or intraplaque hemorrhages, hematoxylin-eosin and fibrin
[Mallory’s phosphotungstic acid-hematoxylin]). Macrophages and
smooth muscle cell stains were analyzed quantitatively by computer-
ized analyses and expressed as a percentage of plaque area. Plaque neo-
vascularization was identified by morphological structure with CD34+
staining that was counted in 3 hotspots and subsequently averaged per
cross-section. Collagen and calcifications were scored semiquantita-
tively at a ×40 magnification, according to the following criteria: no
(1) or minor (2) staining along part of the luminal border of the plaque
or a few scattered spots within the lesion; moderate (3) or heavy (4)
staining was scored when along the entire luminal border or evident
parts within the lesion. Also these categories were grouped into no/
minor and moderate/heavy for logistic regression analyses. Size of the
lipid core was visually assessed using polarized light and cut off at
40% of the plaque area, on the basis of the association with rupture
prone plaques. Luminal thrombus was defined as a solid mass formed
from fibrin and platelets superimposed on the plaque, within the
vascular lumen. Loose erythrocytes in the lumen were scored negative
for luminal thrombi. Plaque thrombosis was defined as the combination
of either the presence of luminal thrombus or intraplaque hemorrhage.
Results are also shown separately. All of the histologic scorings were
performed by the same dedicated technician during the entire study
period. To examine whether the assessment of plaque characteristics
had changed over time, we performed additional intraobserver analyses
between different time intervals of our longitudinal study.

Definitions of Risk Factors and Medical Treatment
At the time of inclusion, all of the patients were asked to complete an
extensive questionnaire, as reported previously. Systolic blood pres-
sure was measured before surgery in the outpatient clinic, together
with height and length to calculate the BMI. Blood withdrawal was
performed at baseline to assess serum total cholesterol and creatinine
levels. Creatinine was used to calculate the estimated glomerular fil-
tration rate with the Modification of Diet in Renal Disease formula. Patients
were considered smokers if they reported to be smoking until
the year of inclusion. Diabetic status was restricted to those patients
receiving medical treatment including insulin or oral glucose-lowering
drugs. Statin use at baseline was registered, as well as the use of antith-
platelet drugs including β-blockers, angiotensin-converting enzyme
inhibitors, angiotensin II antagonists, and diuretics. Furthermore, pre-
scription of antiarrhythmic medication was registered, including aspi-
рин, oral anticoagulants, clopidogrel, and dipyridamole.

Follow-Up and Outcome
The patients included in the Athero-Express study underwent a clini-
cal follow-up that has been described in detail previously. In short,
patients were asked to fill in the follow-up questionnaire after 1, 2,
and 3 years. If the questionnaire was answered positively, further
research was performed to investigate the potential outcome of the
event. If the patient did not respond, the general practitioner was
contacted. The events were assessed by 2 members of an outcome
assessment committee. The composite end point included any death
of presumed vascular origin (stroke, myocardial infarction, sudden
death, or other vascular death), nonfatal stroke, myocardial infar-
tion, or any intervention not planned at the time of inclusion.

Statistical Analyses
Statistical analyses were performed with SPSS 17.0 (SPSS Inc,
Chicago, IL). To avoid the limitation of complete case analyses, mul-
tiple imputation was used to calculate missing values. Table I (avail-
able in the online-only Data Supplement) gives information on the
amount of data missing in our study. To investigate time trends with
sufficient sample sizes in different time periods, we studied plaque
and patient characteristics over 2-year intervals from 2002 to 2011
using χ² statistics. Potential confounding variables were predefined
on the basis of observed baseline incomparability over time (P value
for selection <0.20) and introduced in regression in a stepwise man-
er to estimate their individual contribution in logistic regression
models. For this analysis, plaque characteristics were grouped into
no/minor and moderate/heavy.

Results
Patients
The demographics in the patient population scheduled for
carotid endarterectomy are shown in Table I. The mean age at
the time of surgery was 66.4 (±8.5) years in 2002 through
2003 and 69.9 (±10.1) in 2010 through 2011 (±SD). The propor-
tion of asymptomatic patients in the same period of time
was 21.2% for 2002 through 2003 and 9.2% for 2010 through
2011. For symptomatic patients, the median time between the
last event and surgery was 111 days (range, 54–159 days) in
2002 through 2003 and 20 days (range, 11–35 days) in 2010
through 2011 (Table I). Prevalence of active smoking until
the year of surgery was 45.6% in 2002 through 2003 and 32.1%
in 2010 through 2011. Furthermore, total cholesterol levels at
the time of inclusion were lower in more recent years, con-
comitant with a higher frequency of statin prescription. The
prescription of angiotensin II antagonists and dipyridamole
was also more frequent in more recent years (Table 2). On the
other hand, prescriptions of oral anticoagulants and clopi-
dogrel were highest in the early years of the study. These factors
were introduced in the multivariate model to assess their indi-
vidual contribution to the association between plaque charac-
teristics and year of inclusion.

Plaque Composition
During the past decade, the prevalence of plaques with large
lipid cores, covering >40% of the plaque surface, was frequently
observed in 2002 through 2003 (33.2%) and less frequently
observed in 2010 through 2011 (14.4%; P<0.001; Table 3). The
occurrence of plaque thrombosis was more prevalent in
the early years (74.4%) as compared with more recent years
(37.6%; P=0.001). There were no differences in the number of
intraplaque vessels over the years. The proportion of heavily
calcified plaques was also less prevalent in more recent years.

Strong fluctuations were observed in macrophage and
smooth muscle cell counts between 2002 and 2011. For both
cell types the highest values were observed in 2006 through
2007, and the lowest values were observed in 2010 through 2011. The median macrophage percentages were 0.41% in 2002 through 2003, and 0.09% in 2011 through 2012. Figure 1 shows the semiquantitative data on plaque characteristics that are currently believed to be causally related to the vulnerability of the plaque in 2-year intervals. Figure 1A shows that the decrease in histologically identified plaque thrombosis was mostly attributed to a decrease in both luminal thrombosis and intraplaque hemorrhage. Also, the decrease in plaques with higher fat content coincided with an increase in plaques with no detectable fat (Figure 1B). For macrophages and calcium (Figure 1C and 1D), the heavy staining was less often observed over time, whereas minor staining prevailed in more recent years. Raw scatterplots of macrophages and smooth muscle cells, as well as semiquantitative data on collagen and smooth muscle cells, are depicted in Figure I in the online-only Data Supplement.

The association between plaque characteristics and year of surgery was assessed after adjustment for patient and procedural characteristics that changed over time (age, index event, delay between event and surgery, and medication use) and traditional risk factors that are known to be associated with atherosclerotic disease progression (presence of contralateral internal carotid stenosis >50%, history of peripheral arterial disease, history of coronary arterial disease, smoking, mean arterial pressure, total cholesterol levels, and kidney function [estimated glomerular filtration rate]). After adjustment, all of the aforementioned plaque characteristics, except for smooth muscle cell content, remained significantly associated with year of surgery and indicated a decrease in features that are considered to be associated with carotid plaque instability over time. Per 2-year increase, the adjusted odds ratios for atheromatous plaques, plaque thrombosis, and calcium were 0.72 (95% confidence interval, 0.650-0.789; P <0.001), 0.62 (95% confidence interval, 0.569-0.679; P <0.001), and 0.75 (95% confidence interval, 0.692-0.816, P <0.001), respectively (Figure 2).

Table 1. Patient Characteristics Over Time

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<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>66.4 (8.50)</td>
<td>68.3 (9.3)</td>
<td>68.7 (9.2)</td>
<td>69.7 (8.9)</td>
<td>69.9 (10.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, male, %</td>
<td>69.2</td>
<td>70.3</td>
<td>67.9</td>
<td>67.2</td>
<td>67.2</td>
<td>0.357</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>45.6</td>
<td>33.1</td>
<td>33.8</td>
<td>30.4</td>
<td>32.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>21.2</td>
<td>22.1</td>
<td>25.6</td>
<td>19.3</td>
<td>22.1</td>
<td>0.893</td>
</tr>
<tr>
<td>Mean arterial pressure (SD), mm Hg</td>
<td>107.9 (14.7)</td>
<td>107.9 (15.6)</td>
<td>107.2 (16.3)</td>
<td>107.7 (16.9)</td>
<td>104.5 (15.1)</td>
<td>0.023</td>
</tr>
<tr>
<td>Total cholesterol (SD), mmol/mL</td>
<td>5.2 (1.1)</td>
<td>4.7 (1.2)</td>
<td>4.4 (1.1)</td>
<td>4.4 (1.2)</td>
<td>4.9 (1.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>eGFR (SD), mL/min/1.73 m²</td>
<td>71.5 (18.6)</td>
<td>70.3 (19.8)</td>
<td>72.7 (22.8)</td>
<td>71.3 (20.2)</td>
<td>73.5 (19.9)</td>
<td>0.157</td>
</tr>
<tr>
<td>BMI (SD), kg/m²</td>
<td>26.4 (3.8)</td>
<td>26.2 (3.9)</td>
<td>26.5 (3.7)</td>
<td>26.1 (3.4)</td>
<td>26.7 (4.6)</td>
<td>0.525</td>
</tr>
<tr>
<td>Time since last cerebrovascular event, median (IQR), d</td>
<td>111 (54–159)</td>
<td>65 (26–121)</td>
<td>44 (18–72)</td>
<td>30 (13–53)</td>
<td>20 (11–35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Index event, %</td>
<td>&lt;0.001</td>
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Table 2. Preoperative Medication Prescription Over Time

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<tbody>
<tr>
<td>Statins, %</td>
<td>64.8</td>
<td>73.3</td>
<td>81.2</td>
<td>78.6</td>
<td>79.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>β-Blockers, %</td>
<td>46.0</td>
<td>47.5</td>
<td>44.3</td>
<td>46.7</td>
<td>42.8</td>
<td>0.417</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitors, %</td>
<td>30.0</td>
<td>34.1</td>
<td>33.0</td>
<td>26.8</td>
<td>33.2</td>
<td>0.707</td>
</tr>
<tr>
<td>Angiotensin II antagonists, %</td>
<td>18.4</td>
<td>18.1</td>
<td>22.2</td>
<td>24.1</td>
<td>26.2</td>
<td>0.004</td>
</tr>
<tr>
<td>Diuretics, %</td>
<td>31.2</td>
<td>35.0</td>
<td>35.8</td>
<td>38.3</td>
<td>33.6</td>
<td>0.388</td>
</tr>
<tr>
<td>Aspirin, %</td>
<td>87.6</td>
<td>85.5</td>
<td>84.4</td>
<td>83.7</td>
<td>84.9</td>
<td>0.289</td>
</tr>
<tr>
<td>Oral anticoagulants, %</td>
<td>14.4</td>
<td>11.8</td>
<td>13.6</td>
<td>12.0</td>
<td>9.2</td>
<td>0.137</td>
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eGFR indicates estimated glomerular filtration rate; IQR, interquartile range.
revealed that none of these factors could be considered as confounding variables (change in $\beta < 10\%$). Table II in the online-only Data Supplement shows the odds ratios of the association between plaque and year of inclusion following a stepwise correction for potential confounders. Table III in the online-only Data Supplement shows the adjusted odds ratio for subgroups without statin use, blood pressure medication, and smoking. Table 4 depicts the time-dependent changes in plaque characteristics for each clinical presentation before surgery. The data reveal that the temporal changes in plaque characteristics were consistent among these subgroups. During 3 years of follow-up, event rates for composite cardiovascular end points remained similar. Of those included in 2002–2003, 31.7% had experienced a major cardiovascular end point within 3 years compared with 26.4% of those included in 2008 (Kaplan Meier plots are shown in Figure II in the online-only Data Supplement).

**Discussion**

The current study shows time-dependent changes toward more plaques that show features currently believed to be associated with plaque stability in patients with carotid endarterectomy. This coincided with more favorable cardiovascular risk factor levels and more prescription of medications. Our data do not prove that more favorable risk factor levels or medication in the last decade caused the time-dependent changes in plaque characteristics. We can only conclude that risk factors and plaque composition improved concurrently over the years.

Changes in cardiovascular disease morbidity and mortality in populations over decades have been documented.\textsuperscript{1,2,4,5} For

### Table 3. Presence of Atherosclerotic Plaque Components Over Time

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<tbody>
<tr>
<td>Atheromatous plaque (lipid core &gt;40% of plaque surface), %</td>
<td>33.2</td>
<td>36.0</td>
<td>26.7</td>
<td>21.1</td>
<td>14.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plaque thrombosis, %</td>
<td>74.4</td>
<td>75.5</td>
<td>62.5</td>
<td>49.1</td>
<td>37.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intraplaque vessels, median (IQR)</td>
<td>6.7 (4.3–10.4)</td>
<td>8.5 (5.7–12.3)</td>
<td>7.7 (4.7–11.9)</td>
<td>7.3 (3.7–12.9)</td>
<td>6.3 (2.3–11.7)</td>
<td>0.436</td>
</tr>
<tr>
<td>Collagen rich, %</td>
<td>73.6</td>
<td>85.8</td>
<td>80.1</td>
<td>76.8</td>
<td>78.6</td>
<td>0.643</td>
</tr>
<tr>
<td>Median macrophage staining per plaque area (IQR) %</td>
<td>0.41 (0.09–1.30)</td>
<td>0.46 (0.08–1.23)</td>
<td>1.04 (0.46–1.96)</td>
<td>0.27 (0.10–0.64)</td>
<td>0.09 (0.01–0.51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median smooth muscle cell staining per plaque area (IQR), %</td>
<td>2.03 (0.73–4.34)</td>
<td>1.08 (0.34–2.65)</td>
<td>2.19 (1.00–3.75)</td>
<td>1.23 (0.46–2.21)</td>
<td>0.68 (0.13–1.99)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcified plaque, %</td>
<td>52.0</td>
<td>62.0</td>
<td>59.7</td>
<td>51.2</td>
<td>24.0</td>
<td>&lt;0.001</td>
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IQR indicates interquartile range.

Figure 1. Semiquantitative plaque information on intraplaque hemorrhage and luminal thrombus (A), fat (B), macrophages (C), and calcification (D) based on the presence or degree of staining.
both, stroke and coronary heart disease favorable time trends in incidence and mortality have been reported in high-income countries.1,2,4 The temporal changes are thought to be related to a more favorable risk factor profile in populations both before and after the event. Our study shows similar trends in risk factor levels and medication prescription in patients with carotid endarterectomy from 2002 to 2011. We observed a decrease in total cholesterol, improved mean arterial pressure, and a decrease in the prevalence of smoking in more recent years. Also, an increase in the prescription of statins and angiotensin II receptor antagonists in more recent years was observed. Controversially, the lower number of plaques that show features currently believed to be associated with plaque instability, together with a more favorable risk factor level and increased medication, did not coincide with a shift toward less severe events before the patients were scheduled for carotid endarterectomy in our carotid endarterectomy population. On the contrary, the inclusion of asymptomatic patients in our cohort even decreased over time. In addition, the more recent included patients with carotid endarterectomy demonstrated similar event-free survival in the 3-year follow-up after surgery compared with patients included in the early years of the Athero-Express study. These results show that primary prevention measures may have improved in the last decade coinciding with plaques that show features currently believed to be associated with plaque stability. Yet, this did not translate into a reduced event rate in patients with severe carotid artery disease. The time-dependent changes in plaque characteristics without a subsequent reduction in inclusion rate or secondary manifestations of atherosclerotic disease are of interest because they might challenge the current concept that the unstable vulnerable plaque is the major determinant of outcome in patients experiencing carotid artery disease.

Multiple studies suggest that the stroke population is changing in terms of severity of the disease and stroke subtypes.18-21 A registry study spanning a 25-year time period shows that stroke patients were less likely to experience large artery disease with and without significant stenosis.19 In the Netherlands, a remarkable decline in fatal ischemic stroke has been observed in the last 20 years.18 This trend was not only observed for stroke in the Netherlands but also for coronary heart disease.22

Our data support the idea that the underlying mechanism explaining cerebral manifestations of atherosclerotic disease may be changing. Inevitably, our findings question the validity of the vulnerable plaque hypothesis in this population. The patients with carotid endarterectomy in our study were being referred to the hospital with major events such as stroke also in the more recent years. Yet, their atherosclerotic plaque removed during surgery shows features currently believed to be associated with plaque stability. Although the symptomatic plaques revealed more stable characteristics in recent years, we did not observe secular trends in the proportion of patients with contralateral internal artery stenosis. This suggests that the observed changes in the composition of atherosclerotic plaques come without changes in the extent of atherosclerotic disease.

Changes in patient demographics over time included increasing age, decreasing delay between surgery and the last event, and changes in symptomatic status, because we included less asymptomatic patients. These changes in our population even strengthen the validity of our findings. The older age of patients undergoing carotid endarterectomy during the inclusion period might have several explanations. One might hypothesize that patients experience stroke at an older age, as progression of atherosclerotic disease has been slowed down because of successful improvements in primary prevention.23 On the other hand, overall prognosis and life expectancy of individuals is improving, thereby allowing selection criteria for carotid revascularization to be extended. Finally, elderly patients seem to have increased risks during carotid stenting as compared with younger patients.24-27 These results from large randomized trials over the past years might have resulted in more frequent referral of elderly patients to undergo carotid endarterectomy instead of carotid stenting. Older age has been reported to be associated with plaque characteristics currently believed to be associated with plaque instability.28,29 Controversially, in our study the plaques showed more features considered to reflect stable plaques despite the fact that the patients became older at inclusion. Also, the delay between the last event and surgery has decreased significantly, because the timing of carotid endarterectomy is essential in the prevention of secondary stroke.30 This has resulted in adjustments of guidelines and, in our experience, shortening in referral delay. It has been shown previously that plaques from patients

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### Figure 2. Adjusted odds ratios for the presence of atherosclerotic plaque characteristics, per 2-year increase from 2002 to 2011. Odds ratios and P values were adjusted for age, sex, smoking, total cholesterol, time since last event, index event, statin use, angiotensin II antagonist use, oral anti-coagulant use, clopidogrel use, and dipyriramol use.

<table>
<thead>
<tr>
<th>Plaque characteristic</th>
<th>Adjusted odds ratio [95% CI]</th>
<th>Adjusted P value</th>
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<tbody>
<tr>
<td>Plaque thrombosis</td>
<td>0.62 [0.569-0.679]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atheromatous plaque</td>
<td>0.72 [0.656-0.789]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcified plaque</td>
<td>0.75 [0.692-0.816]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Macrophage rich</td>
<td>0.87 [0.806-0.940]</td>
<td>0.001</td>
</tr>
<tr>
<td>Collagen rich</td>
<td>0.99 [0.877-1.088]</td>
<td>0.515</td>
</tr>
<tr>
<td>Smooth muscle cell rich</td>
<td>1.05 [0.961-1.146]</td>
<td>0.286</td>
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operated on shortly after stroke reveal more inflammatory and thus unstable characteristics, including macrophages and inflammatory proteins. On the basis of these previous observations, one would expect a higher prevalence of plaques with more features associated with plaque instability in patients undergoing carotid surgery. Nevertheless, we observed a stabilization of plaques in the last decade, suggesting that the plaque differences over time are not driven or confounded by

IQR indicates interquartile range.
shortening of the timing of surgery. Carotid endarterectomy for asymptomatic individuals has been debated for a long time and especially in recent years because of improvements in medical treatment. Indications for surgery have likewise been changed over the past decade, and a conservative approach for most asymptomatic patients is now proposed by neurologists and vascular surgeons. Plaques from asymptomatic patients have a more fibrous and less inflammatory phenotype as compared with patients with transient ischemic attack and stroke.33 We observed more plaques with more fibrotic and less inflammatory features, whereas the proportion of asymptomatic patients was lower. Furthermore, our findings are supported by our subgroup analyses that shows that the time-dependent changes in plaque characteristics were very consistent in the well-defined 4 patient groups presenting with a stroke, transient ischemic attack, or ocular symptoms or those who were asymptomatic. We have performed several analyses in which we were unable to detect variables that confounded the relation between inclusion year and plaque phenotype.

Regarding limitations, bias in the study of time-dependent trends may occur when, for example, the diagnosis of the disease has changed. Changes in the detection of the disease may result in the phenomena like Will Rogers (an increased incidence of the disease and lower rates of case fatality).34 We did not observe a decline in the total number of patients included or a shift toward less symptomatic patients in the Athero-Express study. However, we cannot exclude that our observations over time could be attributed to the differences in patient characteristics and indications for surgery that have occurred over this same interval. The observational nature of this study merits careful consideration, and before making causal inferences, this observation requires serial imaging plaque characterization measurements in patients. In addition, the awareness among patients whose mild and transient symptoms may be because of a minor stroke or a transient ischemic attack may have been improved. For example, as in many countries, repetitive educational campaigns were raised in the Netherlands by the Dutch Heart Foundation in the late 1990s focusing on recognition of early signs and symptoms of stroke and transient ischemic attacks. This may have led to the aforementioned inclusion of less-severely diseased patients in our study with concomitant different plaque characteristics. In part of the histologic analyses we applied arbitrary cutoff values to categorize plaque phenotypes. This may have influenced our observations. However, computerized quantitative measurements, such as CD68, also revealed a consistent change toward plaque stabilization over time. In addition, the interpretation of plaque characteristics has been performed by the same dedicated technician over the entire study period to ensure continuity. The scoring system that was used has been validated previously by an additional observer and an independent pathologist.52 Although we cannot fully exclude that changes over time are caused by misinterpretations, we believe that the strong consistency in the data and different stains supports our view that carotid symptomatic lesions show stabilization features over the last decade.

In conclusion, over the past decade, atherosclerotic plaques harvested during carotid endarterectomy show a time-dependent change in plaque composition characterized by a decrease in features currently believed to be causal for plaque instability. This appears to go hand in hand with improvements in risk factor management.

Acknowledgments

Drs Van Lammeren, Den Ruijter, and Pasterkamp had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The funding sponsor was not involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the article. Drs Van Lammeren, de Kleijn, Moll, and Pasterkamp were involved in study concept and design. Drs Van Lammeren, Vrijenhoek, De Borst, and De Vries and Mr van der Laan acquired the data. Drs Van Lammeren, Den Ruijter, Vrijenhoek, and Vink; Mr van der Laan; and Ms Vel lemma analyzed and interpreted the data. Drs Van Lammeren and Den Ruijter drafted the article. Drs Pasterkamp, Moll, Vink, De Vries, de Kleijn, De Borst, and Bots were involved in critical revision of the article for important intellectual content. Drs Van Lammeren, Den Ruijter, and Bots conducted statistical analysis. Drs Pasterkamp, Moll, and de Kleijn obtained funding. Dr Vrijenhoek and Ms Vel lemma were involved with administrative, technical, or material support. Dr Pasterkamp supervised the study.

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Disclosures

None.

References


Time-Dependent Changes in Atherosclerotic Plaque Composition in Patients Undergoing Carotid Surgery

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### Supplemental Table 1

<table>
<thead>
<tr>
<th>Variables used in this study</th>
<th>% missingness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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</tr>
<tr>
<td>Gender</td>
<td>0</td>
</tr>
<tr>
<td>Smoking</td>
<td>4.9</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.7</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>17.5</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>31.5</td>
</tr>
<tr>
<td>eGFR</td>
<td>4.3</td>
</tr>
<tr>
<td>Body mass index</td>
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</tr>
<tr>
<td>Time since last cerebrovascular event</td>
<td>6.6</td>
</tr>
<tr>
<td>Index event</td>
<td>0.7</td>
</tr>
<tr>
<td>Contralateral stenosis</td>
<td>8.9</td>
</tr>
<tr>
<td>History of peripheral artery disease</td>
<td>2.0</td>
</tr>
<tr>
<td>History of coronary artery disease</td>
<td>2.0</td>
</tr>
<tr>
<td>Statins</td>
<td>3.1</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>3.1</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>3.3</td>
</tr>
<tr>
<td>Angiotensin II antagonists</td>
<td>3.4</td>
</tr>
<tr>
<td>Diuretics</td>
<td>3.1</td>
</tr>
<tr>
<td>Aspirin</td>
<td>3.2</td>
</tr>
<tr>
<td>Oral anticoagulants</td>
<td>3.1</td>
</tr>
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Supplemental Table 2: Odds ratios between year of inclusion and plaque phenotype after addition of potential confounders (bold).

<table>
<thead>
<tr>
<th>Odds ratio (95% CI) between year of inclusion (per 2 year increase in time) and</th>
<th>Plaque thrombosis</th>
<th>Atheromatous plaque</th>
<th>Calcified plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate</td>
<td>0.64 (0.59-0.69)</td>
<td>0.76 (0.70-0.83)</td>
<td>0.76 (0.71-0.82)</td>
</tr>
<tr>
<td>Age</td>
<td><strong>0.63 (0.58-0.69)</strong></td>
<td><strong>0.74 (0.68-0.81)</strong></td>
<td><strong>0.75 (0.69-0.81)</strong></td>
</tr>
<tr>
<td>Age, gender</td>
<td>0.63 (0.58-0.69)</td>
<td>0.74 (0.68-0.81)</td>
<td>0.75 (0.69-0.81)</td>
</tr>
<tr>
<td>Age, gender, smoking</td>
<td>0.63 (0.58-0.69)</td>
<td>0.74 (0.68-0.81)</td>
<td>0.75 (0.69-0.81)</td>
</tr>
<tr>
<td>Age, gender, smoking, total cholesterol</td>
<td>0.63 (0.58-0.69)</td>
<td>0.74 (0.67-0.81)</td>
<td><strong>0.74 (0.68-0.80)</strong></td>
</tr>
<tr>
<td>Age, gender, smoking, time since last event</td>
<td>0.63 (0.58-0.69)</td>
<td><strong>0.73 (0.67-0.80)</strong></td>
<td>0.74 (0.69-0.80)</td>
</tr>
<tr>
<td>Age, gender, smoking, time since last event, index event</td>
<td>0.63 (0.57-0.68)</td>
<td>0.73 (0.66-0.80)</td>
<td>0.74 (0.69-0.80)</td>
</tr>
<tr>
<td>Age, gender, smoking, total cholesterol, time since last event, index event, statin use</td>
<td>0.63 (0.58-0.68)</td>
<td>0.73 (0.67-0.80)</td>
<td><strong>0.75 (0.69-0.81)</strong></td>
</tr>
<tr>
<td>Age, gender, smoking, total cholesterol, time since last event, index event, angiotensin II antagonist use</td>
<td>0.63 (0.58-0.69)</td>
<td>0.73 (0.67-0.80)</td>
<td>0.75 (0.69-0.81)</td>
</tr>
<tr>
<td>Age, gender, smoking, total cholesterol, time since last event, index event, angiotensin II antagonist use, oral anticoagulant use</td>
<td>0.63 (0.58-0.69)</td>
<td>0.73 (0.67-0.80)</td>
<td>0.75 (0.69-0.81)</td>
</tr>
<tr>
<td>Age, gender, smoking, total cholesterol, time since last event, index event, statin use, angiotensin II antagonist use, oral anticoagulant use, clopidogrel use</td>
<td>0.63 (0.58-0.69)</td>
<td><strong>0.74 (0.67-0.81)</strong></td>
<td><strong>0.76 (0.70-0.82)</strong></td>
</tr>
<tr>
<td>Age, gender, smoking, total cholesterol, time since last event, index event, statin use, angiotensin II antagonist use, oral anticoagulant use, clopidogrel use, dipyridamol use</td>
<td>0.63 (0.57-0.69)</td>
<td>0.74 (0.67-0.81)</td>
<td>0.76 (0.70-0.82)</td>
</tr>
</tbody>
</table>
Supplemental Table 3. Subgroup analysis, stratified for statin use, blood pressure medication and smoking status.

<table>
<thead>
<tr>
<th></th>
<th>Statin</th>
<th>No statin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plaque thrombosis,</strong></td>
<td><strong>OR (95% CI)</strong></td>
<td><strong>OR (95% CI)</strong></td>
</tr>
<tr>
<td>(95% CI)</td>
<td>0.65 (0.58-0.72)</td>
<td>0.58 (0.48-0.70)</td>
</tr>
<tr>
<td>Atheromatous plaque,</td>
<td>0.77 (0.69-0.86)</td>
<td>0.65 (0.54-0.79)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.75 (0.68-0.82)</td>
<td>0.82 (0.69-0.96)</td>
</tr>
<tr>
<td><strong>Calcified plaque,</strong></td>
<td><strong>OR (95% CI)</strong></td>
<td><strong>OR (95% CI)</strong></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.77 (0.69-0.86)</td>
<td>0.65 (0.54-0.79)</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td><strong>medication</strong></td>
<td><strong>No blood pressure medication</strong></td>
</tr>
<tr>
<td><strong>Plaque thrombosis,</strong></td>
<td><strong>OR (95% CI)</strong></td>
<td><strong>OR (95% CI)</strong></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.54 (0.44-0.66)</td>
<td>0.65 (0.59-0.72)</td>
</tr>
<tr>
<td>Atheromatous plaque,</td>
<td>0.65 (0.51-0.82)</td>
<td>0.76 (0.69-0.85)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.61 (0.50-0.74)</td>
<td>0.81 (0.73-0.89)</td>
</tr>
<tr>
<td><strong>Smoker</strong></td>
<td><strong>Non-smoker</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Plaque thrombosis,</strong></td>
<td><strong>OR (95% CI)</strong></td>
<td><strong>OR (95% CI)</strong></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.64 (0.55-0.74)</td>
<td>0.60 (0.50-0.74)</td>
</tr>
<tr>
<td>Atheromatous plaque,</td>
<td>0.78 (0.66-0.92)</td>
<td>0.72 (0.64-0.81)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.76 (0.66-0.87)</td>
<td>0.71 (0.68-0.83)</td>
</tr>
</tbody>
</table>

Adjusted odds ratios for the presence of atherosclerotic plaque characteristics, per 2 year increase from 2002-2011. Odds ratios and p values were adjusted for age, gender, smoking, total cholesterol, time since last event, index event, statin use (except in the subgroup analyses), angiotensin II antagonist use, oral anticoagulant use, clopidogrel use and dipyridamol use.
Panel A and C show raw scatter plots of the % of macrophages and smooth muscle cells in plaques over the years. The % of plaques that fall into categories of semi-quantitative staining (no, minor, moderate or heavy) are depicted in panel B for collagen and panel D for smooth muscle cells.
Supplemental eFigure 2.

Kaplan Meier survival curves of the patients included in the Athero-Express, stratified by inclusion year.