Gender-associated differences in plaque phenotype of patients undergoing carotid endarterectomy

Willem E. Hellings, MD,[a,b] Gerard Pasterkamp, MD, PhD,[b] Bart A. N. Verhoeven, MD, PhD,[a]
Dominique P. V. De Kleijn, PhD,[b] Jean-Paul P. M. De Vries, MD, PhD,[c] Kees A. Seldenrijk, MD, PhD,[d]
Theo van den Broek, MD,[b] and Frans L. Moll, MD, PhD,[a] *Utrecht and Nieuwegein, The Netherlands*

Background: Carotid endarterectomy to prevent a stroke is less beneficial for women compared with men. This benefit is lower in asymptomatic women compared with asymptomatic men or symptomatic patients. A possible explanation for this gender-associated difference in outcome could be found in the atherosclerotic carotid plaque phenotype. We hypothesize that women, especially asymptomatic women, have more stable plaques than men, resulting in a decreased benefit of surgical plaque removal.

Methods: Carotid endarterectomy specimens of 450 consecutive patients (135 women, 315 men) were studied. The culprit lesions were semi-quantitatively analyzed for the presence of macrophages, smooth muscle cells, collagen, calcifications, and luminal thrombus. Plaques were categorized in three phenotypes according to overall presentation and the amount of fat. Protein was isolated from the plaques for determination of interleukin-6 (IL-6) and IL-8 concentrations and matrix metalloproteinase-8 (MMP-8) and MMP-9 activities.

Results: Atheromatous plaques (>40% fat) were less frequently observed in women than in men (22% vs 40%; $P < .001$). In addition, plaques obtained from women more frequently revealed low macrophage staining (11% vs 18%; $P = .05$) and strong smooth muscle cell staining (38% vs 24%; $P = .001$). Compared with men, women had a lower plaque concentration of IL-8 ($P = .001$) and lower MMP-8 activity ($P = .01$). The observed differences were most pronounced in asymptomatic women, who showed the most stable plaques, with an atheromatous plaque in only 9% of cases compared with 39% in asymptomatic men ($P = .02$). In addition, a large proportion of plaques obtained from asymptomatic women showed high smooth muscle cell content (53% vs 30%; $P = .03$) and high collagen content (55% vs 24%; $P = .003$). All relations between gender and plaque characteristics, except for MMP-8, remained intact in a multivariate analysis, including clinical presentation and other cardiovascular risk factors.

Conclusion: Carotid artery plaques obtained from women have a more stable, less inflammatory phenotype compared with men, independent of clinical presentation and cardiovascular risk profile. Asymptomatic women demonstrate the highest prevalence of stable plaques. These findings could explain why women benefit less from carotid endarterectomy compared with men. (J Vasc Surg 2007;45:289-97.)

Carotid endarterectomy (CEA) reduces the risk of stroke in both symptomatic and asymptomatic patients with high-grade carotid artery stenosis. The benefit of the operation in terms of stroke reduction differs among patient subgroups. Gender is a major determinant of the long-term outcome after carotid surgery.

It has been well established that CEA is more beneficial for men than for women. Carotid plaque associated stroke risk in patients on best medical treatment is higher in men than in women. After carotid surgery, stroke risk is reduced to comparable levels for both sexes, resulting in a larger reduction of stroke risk in men.[1-3] This gender difference in outcome after CEA is evident in symptomatic and asymptomatic patients. Randomized trials suggest that although asymptomatic women still benefit from CEA, their benefit

is smaller compared with asymptomatic men or symptomatic patients.[1-2,4]

Our understanding of these gender differences is incomplete. Different hypotheses have been raised that might account for the observed differences in outcome after carotid endarterectomy between men and women. Duplex analyses of the carotid artery before surgery have demonstrated that plaque volume is larger in men than in women at a comparable stenosis grade and that the plaque size is a predictor of clinical outcome.[5] Effects of hormones on atherosclerosis are becoming better known with increasing research,[6] but no direct pathophysiologic link has been recognized between hormones and outcome of carotid surgery.

The observed clinical differences may also be a direct reflection of carotid plaque characteristics. If women have plaques that are less prone to cause a stroke owing to distal embolization, then removal of such a plaque would be less beneficial. In coronary circulation, certain plaque characteristics are strongly associated with unstable clinical presentation. The vulnerable plaque that gives rise to myocardial infarction or unstable angina is defined as a plaque with high fat content, low structural components (thin fibrous cap, low smooth muscle cell and collagen content), high inflammatory cell content, and increased protease activity.[7-9]

Recent large studies of endarterectomy specimens have shown that the pathophysiology of carotid artery disease is very similar to coronary artery disease. The vulnerable plaque characteristics known from coronary circulation have been linked to symptomatic presentation of carotid artery disease.[10-12] In addition, the association between plaque destabilization and matrix metalloproteinase-8 (MMP-8) and MMP-9 activity in carotid artery plaques has been reported.[13,14]

In this study we hypothesized that women who have been diagnosed with hemodynamically significant atherosclerotic carotid artery disease have more stable carotid plaques than men and that this is especially evident in women who are asymptomatic. This could explain the observation that CEA is less beneficial in women.

PATIENTS AND METHODS

Athero-Express biobank. The Athero-Express is an ongoing longitudinal biobank study with the objective of studying the relation between plaque characteristics and the occurrence of future cardiovascular events.[15] All patients undergoing CEA in two participating Dutch hospitals are asked to participate in the study, with an inclusion rate of 94.6%. At baseline, patients complete an extensive questionnaire and blood is drawn and stored at −80°C.

During CEA, the plaque is transferred to the laboratory and processed and stored according to a standardized protocol. After surgery, patients undergo duplex and clinical follow-up. The Medical Ethical Committees of the participating hospitals have approved the study, and all patients provided written informed consent. For the purpose of the current research question, we studied all consecutive patients undergoing CEA who were included in the Athero-Express study between April 2002 and November 2005.

Patient inclusion and preoperative work-up. The indication for CEA was based on the recommendations published by the Asymptomatic Carotid Surgery Trial (ACST) for asymptomatic patients and European Carotid Surgery Trial (ECST)/North American Symptomatic Carotid Endarterectomy Trial for symptomatic patients (NASCET).[2,16-18] All patients were examined by a neurologist for assessment of their preoperative neurologic status. The percentage of stenosis was determined with duplex ultrasonography, using duplex criteria as described by Strandness et al.[19,20] If the duplex investigation was not conclusive, an additional imaging technique (magnetic resonance angiography, computed tomography angiography, conventional angiography) was used to determine the level of carotid stenosis. Excluded were patients with a terminal malignancy and those who were referred back to a hospital outside The Netherlands immediately after surgery.

Baseline characteristics. Baseline data were obtained by chart review and from extensive questionnaires completed by the participating patients that included questions on history of cardiovascular disease, cardiovascular risk factors (smoking, hypertension, diabetes), and use of medication. Presenting symptoms and duplex stenosis were retrieved from patient charts. Symptom categories were asymptomatic, de-

fined as no carotid territory ischemic symptoms; amaurosis fugax, defined as ipsilateral mono-ocular blindness of acute onset lasting <24 hours; cerebral transient ischemic attack (TIA), defined as ipsilateral focal neurologic deficit of acute onset lasting <24 hours; and ipsilateral stroke. Lipid spectra were determined in blood specimens drawn at baseline.

Carotid endarterectomy. CEA was performed under general anesthesia. Patients received 5000 IU heparin intravenously before cross-clamping. All endarterectomies were performed by an open, noneversion technique, with careful dissection of the bifurcation into the internal and external carotid arteries. The atherosclerotic plaque was immediately transferred to the laboratory after removal.

Plaque processing. The atherosclerotic plaque was dissected into 5-mm segments by a dedicated technician. The segment having the greatest plaque area was defined as the culprit lesion. This segment was fixated in formaldehyde 4%, decalcified for 1 week in ethylenediaminetetraacetic acid, and embedded in paraffin. The other segments were snap frozen in liquid nitrogen and stored at −80°C. Sections of 5-μm thickness were cut on a microtome for immunohistochemical staining.

Plaques were characterized for macrophage content (CD68 staining), smooth muscle cell content (α-actin staining), collagen content (picrosirius red), and extent of calcification (hematoxylin and eosin [H&E] staining) and were analyzed semi-quantitatively and scored as *no*, *minor*, *moderate*, and *heavy* staining, as reported previously.[15] Briefly, *no* and *minor* represent absent or minimal staining with few clustered cells, whereas *moderate* and *heavy* represent larger areas of positive staining. Presence of luminal thrombus (H&E and elastin van Gieson staining) was scored as *absent* or *present*. The percentage of atheroma of the total area of the plaque was visually estimated using the picrosirius red and H&E stains. Three overall phenotypes were considered according to overall presentation and visual estimation of the percentage of atheroma in the plaques: fibrous plaques containing <10% fat, fibroatheromatous, 10% to 40%; or atheromatous, >40% fat.

The scorings were done by observers blinded for patient characteristics. In addition, quantitative measurements were performed for macrophage and smooth muscle cell staining. For this purpose, images of plaque cross-sections were recorded onto a computer workstation using a microscope equipped with a digital camera. The images were captured and analyzed with AnalySIS 3.2 software (Soft Imaging System GmbH, Münster, Germany). The quantification was done as follows: in each plaque, three representative areas were defined and selected in such a way that no media (which was present in the specimen, in most cases) was included. The positive staining in these areas was measured as a percentage of total plaque area using AnalySIS software. The mean of these three measurements was used for further analysis.

Interleukin and matrix metalloproteinase measurements. The segment adjacent to the culprit lesion was used for protein isolation. This frozen segment was mechanically crushed in liquid nitrogen with a pestle and mortar. The

protein isolation was done in two ways: (1) by using Tri-pure reagent (Boehringer Mannheim, Germany), according to the manufacturer's protocol and (2) by dissolving in 40 mM Tris-HCl (pH, 7.5) at 4°C.

Protein from 301 plaques was available for analysis. Interleukin-6 (IL-6) and IL-8 concentrations were determined in all samples. The measurements were done on the Tris-solated samples using a multiplex suspension array system according to the manufacturer's protocol (Bio-Rad Laboratories, Hercules, Calif). MMP-8 and MMP-9 activities were measured for a randomly selected group of 133 patients in Tripure isolated protein using Biotrak activity assays RPN 2635 and RPN 2634, respectively (Amersham Biosciences, Buckinghamshire, UK). MMP activities were expressed as an arbitrary unit. All measurements were done by investigators blinded for patient characteristics.

Data analysis. The statistical software SPSS 11.5 (SPSS Inc, Chicago, Ill) was used for data analysis. Continuous baseline variables are given as mean and standard deviation. All plaque measurements are expressed as median and interquartile range. Equal distribution of baseline variables was determined using the χ^2 test for discrete variables, the Student's t test for normally distributed continuous variables and the Mann Whitney U test for nonnormally distributed continuous variables. The Mann Whitney U test was used for comparison of plaque stainings, IL, and MMP levels between men and women and between combined groups according to gender and symptom status. The association between gender and plaque characteristics was adjusted for traditional cardiovascular risk factors, clinical presentation, and all baseline variables showing an association ($P < .20$) with gender, using multivariate logistic regression. For this purpose ordinal variables were dichotomized into two categories (no/minor staining vs moderate/heavy staining) and continuous variables were dichotomized at the median. Values of $P < .05$ were considered statistically significant.

RESULTS

A total of 450 carotid plaques were obtained. The baseline characteristics are given in Table I. Clinical presentation was equal for men and women: 25% of women and 22% of men were asymptomatic. Women (n = 135) had higher total cholesterol, accompanied by a higher low-density-lipoprotein cholesterol as well as higher high-density-lipoprotein cholesterol. Use of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins), aspirin, and oral anticoagulants did not differ between men and women. The other baseline characteristics, including duplex stenosis, were also comparable.

The plaques obtained from women demonstrated a more fibrous phenotype compared with those obtained from men. Atheromatous plaques were present in 22% of women compared with 40% of men ($P < .001$; Table II). In 38% of women, a heavy smooth muscle cell staining was present, compared with 24% in men ($P = .001$). Heavy macrophage staining was found in 14% of women compared with 21% in men ($P = .05$). Luminal thrombus,

Table I. Baseline characteristics of patients undergoing carotid endarterectomy

Variable*	Women, n (%)	Men, n (%)	P
Patients (n)	135	315	
Age (year)	66.2 ± 9.3	67.7 ± 8.5	.09
Hypertension	100 (74)	211 (67)	.15
Diabetes	30 (22)	62 (20)	.53
Prior intervention			
Vascular	41 (30)	128 (41)	.04†
Ipsilateral carotid	9 (7)	11 (4)	.14
Smoking	41 (31)	76 (25)	.20
Hypercholesterolemia	68 (62)	156 (62)	.96
HRT	6 (7)		
Statins	79 (66)	182 (63)	.58
Aspirin	106 (85)	249 (85)	.90
Oral anticoagulation	17 (14)	48 (16)	.46
NSAID	8 (6)	12 (4)	.32
Cholesterol, mmol/L	5.4 ± 1.1	4.9 ± 1.2	.006†
HDL, mmol/L	1.3 ± 0.36	1.1 ± 0.35	.004†
LDL, mmol/L	3.2 ± 1.0	2.9 ± 1.0	.04†
Triglycerides, mmol/L	2.1 ± 1.2	2.1 ± 1.0	.96
Symptoms			
Asymptomatic	33 (25)	69 (22)	
Amaurosis fugax	18 (13)	45 (14)	.91
TIA	47 (35)	118 (38)	
Stroke	37 (27)	83 (26)	
Duplex stenosis			
50% to 64%	3 (2)	9 (3)	
65% to 89%	46 (36)	112 (37)	.84
90% to 100%	79 (62)	181 (60)	

HRT, Hormone replacement therapy; *NSAID,* nonsteroidal anti-inflammatory drug; *HDL,* high-density lipoprotein; *LDL,* low-density lipoprotein; *TIA,* transient ischemic attack.
*Categoric variables are presented as n (%); continuous variables as mean ± standard deviation.
†Statistically significant ($P < .05$).

calcifications, and collagen staining did not differ consistently between men and women. Assessment of lipid spectra in relation to overall plaque phenotype did not reveal any associations (data not shown).

Plaque characteristics were compared between men and women within the symptom groups (asymptomatic vs TIA/stroke). This analysis shows that differences in plaque characteristics were comparable or even more evident in asymptomatic male and female patients (Fig). Plaque overall phenotype was more atheromatous in asymptomatic men than in asymptomatic women. Atheromatous plaques were found in 9% of asymptomatic women compared with 39% of asymptomatic men ($P = .02$) and 44% of symptomatic men ($P < .001$). This difference was also evident but less prominent when symptomatic women were compared with symptomatic men (27% vs 44%; $P = .003$).

Smooth muscle cell staining also revealed strong differences within the asymptomatic group. High staining for smooth muscle cells was observed in 53% of asymptomatic women compared with 30% of asymptomatic men ($P = .03$) and 20% of symptomatic men ($P < .001$). High collagen staining was present in 53% of asymptomatic women compared with 22% of asymptomatic men ($P = .003$) and 15% of

Table II. Comparison of carotid plaque histology between men and women

	Women* (%)	Men* (%)	P (univariate)	P (multivariate)[†]
Overall phenotype (semi-quantitative)			<.001[‡]	.006[‡]
Fibrous	40.7	24.4		
Fibroatheromatous	37.8	35.2		
Atheromatous	21.5	40.3		
Luminal thrombus			.26	.45
No	77.0	70.6		
Yes	23.0	29.4		
Macrophages (semi-quantitative)			.05[†]	.20
No	18	10.6		
Minor	31.6	33.2		
Moderate	36.8	35.2		
Heavy	13.5	21		
Macrophages (quantitative)			.12	.36
Median area %	0.26	0.39		
IQR	(0.08-0.95)	(0.07-1.30)		
SMC (semi-quantitative)			.001[‡]	.01[‡]
No	0.8	1.9		
Minor	20.3	32.9		
Moderate	41.4	41.9		
Heavy	37.6	24.2		
SMC (quantitative)			.03[‡]	.03[‡]
Median area %	2.27	1.62		
IQR	(0.86-4.35)	(0.47-3.58)		
Collagen (semi-quantitative)			.08	.39
No	0.0	0.3		
Minor	19.4	22.6		
Moderate	53.0	57.6		
Heavy	27.6	19.4		
Calcifications (semi-quantitative)			.41	.88
No	29.6	25.7		
Minor	15.6	24.8		
Moderate	28.9	31.1		
Heavy	25.9	18.4		

SMC, Smooth muscle cells; *IQR,* interquartile range.
*Data presented as percentage or median (IQR), as noted.
[†]Adjusted for symptom status, age, hypertension, diabetes, smoking, prior vascular intervention, prior ipsilateral carotid intervention, and cholesterol levels.
[‡]Statistically significant ($P < .05$).

symptomatic men ($P < .001$). No gender-related difference in collagen staining was observed in the symptomatic patient group. Macrophage staining was not significantly different between men and women within the asymptomatic or symptomatic patient group.

The differences in plaque histology between men and women were paralleled by the inflammatory and protease activity in atherosclerotic plaques (Table III). Women showed lower values of the proinflammatory cytokine IL-8 compared with men (25.9 vs 51.3 pg/mL; $P = .001$). Levels of proinflammatory cytokine IL-6 were not different between men and women. Protease activity was lower in women, with MMP-8 showing significantly lower values than in men (4.2 vs 7.1; $P = .01$), whereas MMP-9 activity was lower without reaching statistical significance (1.6 vs 2.6; $P = .07$). These differences were still present when men and women were subdivided into symptomatic and asymptomatic groups (Table IV). Asymptomatic women showed lower levels of interleukins and MMPs compared with the other groups: IL-8 levels (14.1 vs 83.3 pg/mL; $P < .001$), MMP-8 activity (2.6 vs 9.2; $P = .003$), and

MMP-9 activity (1.1 vs 2.9; $P = .002$) were significantly decreased compared with symptomatic men.

All associations between gender and plaque phenotype, except MMP-8, which were significant in univariate analysis, were also significant when adjusting for symptom status, age, hypertension, diabetes, smoking, prior vascular intervention, prior ipsilateral carotid intervention, and cholesterol levels. This suggests that the observed gender-associated differences in plaque characteristics are not caused by differences in cardiovascular risk factors or clinical presentation.

DISCUSSION

The main finding of this study is that women undergoing CEA have more stable plaques compared with men. Plaques obtained from women contain less fat and macrophages and more smooth muscle cells. This is accompanied by lower IL-8 content and lower MMP-8 activity.

It has been recognized that histologic plaque characteristics are related to the clinical presentation of atherosclerotic coronary and carotid artery disease. To our knowl-
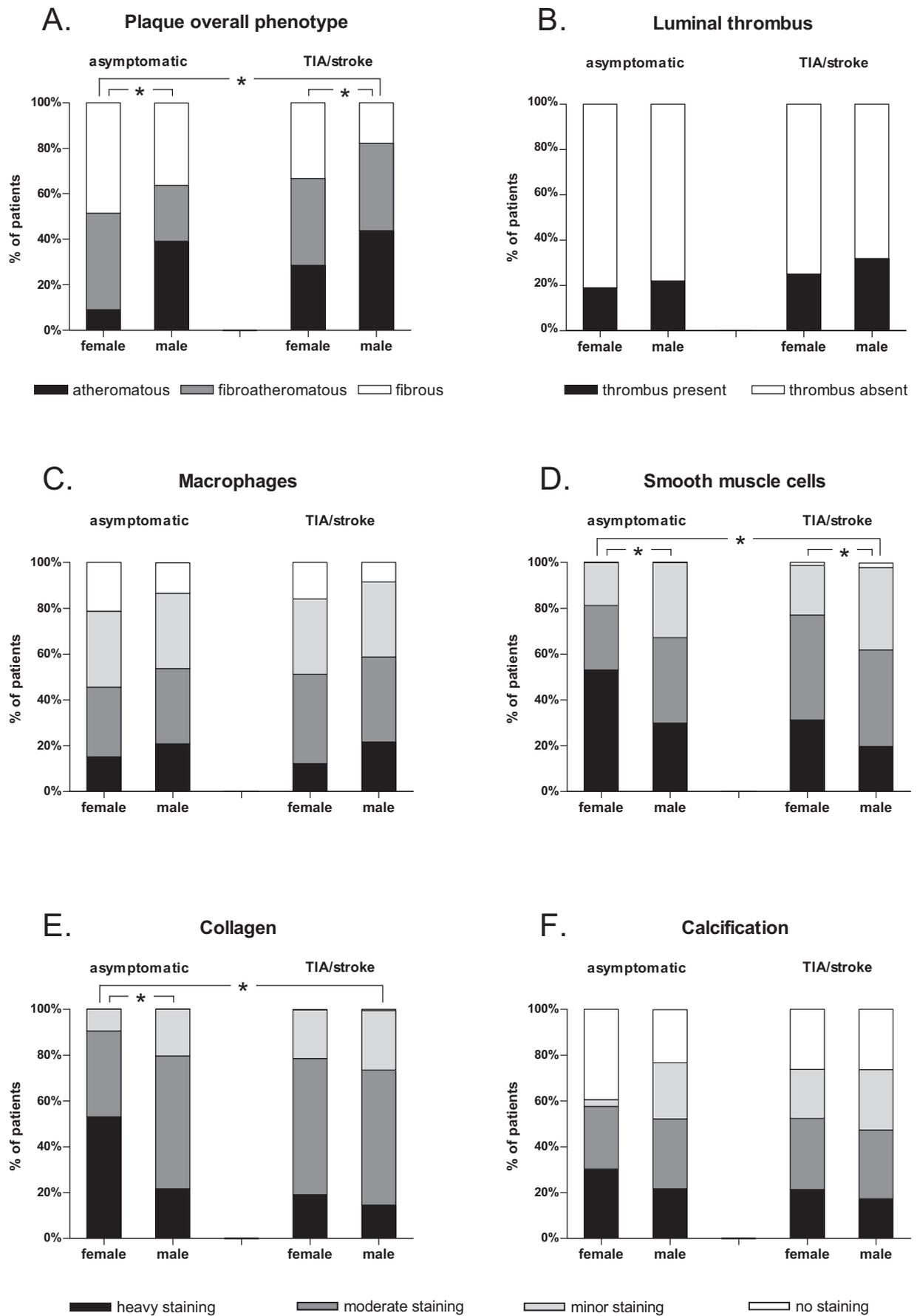
A. Plaque overall phenotype
asymptomatic   TIA/stroke

female male   female male

■ atheromatous ■ fibroatheromatous □ fibrous

B. Luminal thrombus
asymptomatic   TIA/stroke

female male   female male

■ thrombus present □ thrombus absent

C. Macrophages
asymptomatic   TIA/stroke

female male   female male

D. Smooth muscle cells
asymptomatic   TIA/stroke

female male   female male

E. Collagen
asymptomatic   TIA/stroke

female male   female male

F. Calcification
asymptomatic   TIA/stroke

female male   female male

■ heavy staining ■ moderate staining □ minor staining □ no staining

Fig. Comparison of carotid plaque histology between men and women, subdivided by symptom status: asymptomatic vs transient ischemic attack/stroke *(TIA)*. **A,** Plaque overall phenotype. **B,** Luminal thrombus. **C,** Macrophages. **D,** Smooth muscle cells. **E,** Collagen. **F,** Calcification. *$P < .05$.

Table III. Interleukin and matrix metalloproteinase measurements in the plaque compared between men and women

	Women	Men	P (univariate)	P (multivariate)*
IL-6			.2	.89
Median	6.7	8.8		
IQR	0.5-21.1	3.6-19.3		
IL-8			.001†	.01†
Median	25.9	51.3		
IQR	0-59.3	8.8-147.4		
MMP-8			.01†	.34
Median	4.2	7.1		
IQR	1.2-8.1	3.7-11.4		
MMP-9			.07	.42
Median	1.6	2.6		
IQR	0.9-3.1	0.8-6.4		

IL, Interleukin; *IQR,* interquartile range; *MMP,* matrix metalloproteinase.
*Adjusted for symptom status, age, hypertension, diabetes, smoking, prior vascular intervention, prior ipsilateral carotid intervention, and cholesterol levels.
†Statistically significant ($P < .05$).

edge, no study has reported on gender differences in carotid plaque phenotype, probably because of the number of patients required. In coronary artery disease, gender-associated differences in plaque morphology have been described that point to a higher prevalence of fresh thrombus and plaque rupture in men.[21,22] These studies did not, however, specifically address gender-related differences in plaque phenotype but focused on changes in plaque morphology in relation to clinical syndromes like acute myocardial infarction or coronary death.

Our present study indicates that women operated on for carotid artery disease show more stable atherosclerotic plaques than men. These results suggest that not just plaque volume but also plaque phenotype may be associated with adverse outcomes. Iemolo et al[5] found that outward remodeling of the carotid artery was more evident in men than in women. This outward remodeling was predictive of stroke and other cardiovascular events. Outward remodeling has previously been associated with unstable plaque characteristics, rendering the results from Iemolo et al very consistent with ours.[23]

IL-8 was significantly higher in men compared with women, but IL-6 showed no statistically significant difference. Most reduced levels of both cytokines were observed in the asymptomatic women. Both proinflammatory cytokines can be produced by a variety of cells within the atherosclerotic plaque and play an important role in atherosclerosis.[24] It remains to be elucidated whether a differential effect exists between IL-6 and IL-8, which could explain the fact that IL-8 has a stronger relation to gender and plaque instability than IL-6.

In the current study, women showed significantly lower MMP-8 activity in their plaques than did men. MMP-9 was lower in asymptomatic women compared with symptomatic men. Experimental models and previous human end-

arterectomy series have shown that the presence of these MMPs contributes to plaque instability.[13,14] MMPs are important in cell migration, degradation of the fibrous cap, expansive remodeling, and intraplaque neovessel formation.[25] Their production can be directly inhibited by statins.[26]

To our knowledge, no gender differences in MMP activity have been described in human atherosclerotic disease before. Two studies on rat aortas show higher MMP-9 production in male rat aortas compared with females, which could be partially reversed by estrogen treatment or transplantation of the artery into a female rat.[27,28] This suggests that there may be a direct effect of sex hormones on MMP production contributing to attenuation of atherosclerotic disease in females. It is difficult to extrapolate these findings to our population because most of the women in our study cohort are postmenopausal. The observed differences in plaque phenotype could be due to estrogen exposure earlier in life resulting in a more stable plaque phenotype with inherent lower MMP-8 and MMP-9 activity compared with unstable plaques.

The gender-related differences we observed in plaque histology, inflammation, and protease activity were evident within all symptom categories. Therefore, the gender differences cannot merely be explained by different clinical presentation of the carotid artery disease; neither can they be attributed to differences in cardiovascular risk profiles, because all associations between gender and plaque characteristics, except MMP-8, remained intact when correcting for cardiovascular risk factors. The differences are most pronounced in the asymptomatic group: Among asymptomatic women, the prevalence of stable plaques is very high. Although speculative, one of the reasons for the large differences within the asymptomatic group compared with the symptomatic group is that when a plaque becomes symptomatic, some features have already changed, both in men and women. The asymptomatic group probably better represents the true gender-associated differences in plaque phenotype. Nevertheless, we also found gender-associated differences in the symptomatic patient group.

The more prevalent stable plaque phenotype found in women may explain why CEA is less effective in preventing a stroke in women. In all randomized, controlled trials of CEA, women have a lower baseline risk of stroke compared with men that is reduced to an equal level for both sexes after surgery. Thus, women benefit less from CEA then men.[1,2,16-18,29] Asymptomatic women benefit the least from CEA, as shown in the ACST and Asymptomatic Carotid Atherosclerosis Study trials.[1,2,4] Stable, fibrous plaques are less prone to cause ischemic events in the coronary and the carotid arteries.[7-12]

Our observations led us to hypothesize that removal of a stable plaque is less beneficial than removal of a vulnerable plaque, which is more frequently found in asymptomatic men and symptomatic patients. In addition, the aforementioned studies showed that women have a slightly higher perioperative risk than men, also contributing to gender differences in benefit of CEA. Interestingly, the presence of

Table IV. Interleukin and matrix metalloproteinase measurements in the plaque compared between men and women, subdivided by symptom status

	1 *Asymptomatic women*	*2* *Asymptomatic Men*	*3* *Symptomatic women*	*4* *Symptomatic Men*	*1 vs 2*	*3 vs 4*	*1 vs 4*
IL-6					.81	.56	.23
Median	6.3	7.9	11.0	10.3			
IQR	1.6-23.4	3.6-12.0	1.0-23.8	3.9-23.8			
IL-8					.18	.01*	<.001*
Median	14.1	17.2	41.9	83.3			
IQR	0-31.6	0.5-53.6	7.5-90.8	25.6-178.7			
MMP-8					.18	.04*	.003*
Median	2.6	5.0	5.7	9.2			
IQR	0-6.6	2.5-8.5	2.1-8.8	4.5-12.7			
MMP-9					.19	.12	.002*
Median	1.1	1.4	1.7	2.9			
IQR	0.3-2.7	0.4-3.6	1.0-3.7	1.2-7.0			

IL, Interleukin; *IQR,* interquartile range; *MMP,* matrix metalloproteinase.
*Statistically significant ($P < .05$).

a fibrous plaque is associated with an increased amount of microembolization during CEA.[30]

Our results suggest that selecting patients for CEA on the basis of plaque characteristics may hold a promise for the future. This is especially true for patient groups with a small margin of benefit from the operation. We show that variations in plaque phenotype that exist within different patient groups are consistent with previously reported outcomes after CEA. Selection of asymptomatic women for CEA who have vulnerable, unstable plaques might improve the long-term outcome. High-risk groups that benefit greatly from CEA, such as symptomatic men with high-grade stenosis, would probably benefit to a lesser extent from such a strategy. The new imaging techniques such as high-resolution magnetic resonance imaging, single photon emission computed tomography, and palpography may bring the potential of the observed differences in plaque level into clinical practice.[31-33]

Limitations. In the current study, we examined the segment with the largest plaque area and not the entire plaque. The rationale for this method is that the segment of the plaque with the largest plaque burden is the part with the most inflammation and the largest atheroma.[23] It has also been shown that assessment of the culprit segment is reasonably representative for the plaque as a whole.[34] In some cases, an important feature might be missed when only the culprit lesion is studied, potentially masking differences between groups. This drawback is overcome by the large number of patients in our study.

CONCLUSION

Women undergoing CEA have more stable carotid plaques than men, with lower fat, lower macrophage and higher smooth muscle cell content, and lower inflammatory and protease activity. This is not explained by clinical presentation and cardiovascular risk factors, suggesting an independent gender-related effect on carotid plaque phenotype of patients undergoing carotid endarterectomy. The gender-associated differences in plaque phenotype are most evident in asymptomatic women, which could explain why especially asymptomatic women have lower long-term stroke reduction after carotid endarterectomy.

REFERENCES

1. Endarterectomy for asymptomatic carotid artery stenosis. Executive Committee for the Asymptomatic Carotid Atherosclerosis Study. JAMA 1995;273:1421-8.
2. Halliday A, Mansfield A, Marro J, Peto C, Peto R, Potter J, et al. Prevention of disabling and fatal strokes by successful carotid endarterectomy in patients without recent neurological symptoms: randomised controlled trial. Lancet 2004;363:1491-502.
3. Rothwell PM, Eliasziw M, Gutnikov SA, Warlow CP, Barnett HJ. Endarterectomy for symptomatic carotid stenosis in relation to clinical subgroups and timing of surgery. Lancet 2004;363:915-24.
4. Rothwell PM. ACST: which subgroups will benefit most from carotid endarterectomy? Lancet 2004;364:1122-3.
5. Iemolo F, Martiniuk A, Steinman DA, Spence JD. Sex differences in carotid plaque and stenosis. Stroke 2004;35:477-81.
6. Mendelsohn ME, Karas RH. Molecular and cellular basis of cardiovascular gender differences. Science 2005;308:1583-7.
7. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. Circulation 2003;108: 1664-72.

8. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. Circulation 2003;108: 1772-8.

9. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arterioscler Thromb Vasc Biol 1995;15:1512-31.

10. Verhoeven B, Hellings WE, Moll FL, de Vries JP, de Kleijn DP, de Bruin P, et al. Carotid atherosclerotic plaques in patients with transient ischemic attacks and stroke have unstable characteristics compared with plaques in asymptomatic and amaurosis fugax patients. J Vasc Surg 2005;42:1075-81.

11. Redgrave JN, Lovett JK, Gallagher PJ, Rothwell PM. Histological assessment of 526 symptomatic carotid plaques in relation to the nature and timing of ischemic symptoms: the Oxford plaque study. Circulation 2006;113:2320-8.

12. Spagnoli LG, Mauriello A, Sangiorgi G, Fratoni S, Bonanno E, Schwartz RS, et al. Extracranial thrombotically active carotid plaque as a risk factor for ischemic stroke. JAMA 2004;292:1845-52.

13. Loftus IM, Naylor AR, Goodall S, Crowther M, Jones L, Bell PR, et al. Increased matrix metalloproteinase-9 activity in unstable carotid plaques. A potential role in acute plaque disruption. Stroke 2000;31: 40-7.

14. Molloy KJ, Thompson MM, Jones JL, Schwalbe EC, Bell PR, Naylor AR, et al. Unstable carotid plaques exhibit raised matrix metalloproteinase-8 activity. Circulation 2004;110:337-43.

15. Verhoeven BA, Velema E, Schoneveld AH, de Vries JP, de BP, Seldenrijk CA, et al. Athero-express: differential atherosclerotic plaque expression of mRNA and protein in relation to cardiovascular events and patient characteristics. Rationale and design. Eur J Epidemiol 2004;19: 1127-33.

16. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators. N Engl J Med 1991;325:445-53.

17. Randomised trial of endarterectomy for recently symptomatic carotid stenosis: final results of the MRC European Carotid Surgery Trial (ECST). Lancet 1998;351:1379-87.

18. Barnett HJ, Taylor DW, Eliasziw M, Fox AJ, Ferguson GG, Haynes RB, 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; 339:1415-25.

19. Knox RA, Breslau PJ, Strandness DE. A simple parameter for accurate detection of severe carotid disease. Br J Surg 1982 69:230-3.

20. Moneta GL, Edwards JM, Papanicolaou G, Hatsukami T, Taylor LM Jr, Strandness DE, et al. Screening for asymptomatic internal carotid artery stenosis: duplex criteria for discriminating 60% to 99% stenosis. J Vasc Surg 1995;21:989-94.

21. Rittersma SZ, van der Wal AC, Koch KT, Piek JJ, Henriques JP, Mulder KJ, et al. Plaque instability frequently occurs days or weeks before occlusive coronary thrombosis: a pathological thrombectomy study in primary percutaneous coronary intervention. Circulation 2005;111: 1160-5.

22. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 2000;20:1262-75.

23. Pasterkamp G, Schoneveld AH, van der Wal AC, Haudenschild CC, Clarijs RJ, Becker AE, et al. Relation of arterial geometry to luminal narrowing and histologic markers for plaque vulnerability: the remodeling paradox. J Am Coll Cardiol 1998;32:655-62.

24. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352:1685-95.

25. Chase AJ, Newby AC. Regulation of matrix metalloproteinase (matrixin) genes in blood vessels: a multi-step recruitment model for pathological remodelling. J Vasc Res 2003;40:329-43.

26. Luan Z, Chase AJ, Newby AC. Statins inhibit secretion of metalloproteinases-1, -2, -3, and -9 from vascular smooth muscle cells and macrophages. Arterioscler Thromb Vasc Biol 2003;23: 769-75.

27. Woodrum DT, Ford JW, Ailawadi G, Pearce CG, Sinha I, Eagleton MJ, et al. Gender differences in rat aortic smooth muscle cell matrix metalloproteinase-9. J Am Coll Surg 2005;201:398-404.

28. Ailawadi G, Eliason JL, Roelofs KJ, Sinha I, Hannawa KK, Kaldjian EP, et al. Gender differences in experimental aortic aneurysm formation. Arterioscler Thromb Vasc Biol 2004;24:2116-22.

29. Chambers B, Donnan G, Chambers B. Carotid endarterectomy for asymptomatic carotid stenosis. Cochrane Database Syst Rev 2005; CD001923.

30. Verhoeven BA, de Vries JP, Pasterkamp G, Ackerstaff RG, Schoneveld AH, Velema E, et al. Carotid atherosclerotic plaque characteristics are associated with microembolization during carotid endarterectomy and procedural outcome. Stroke 2005;36:1735-40.

31. Davies JR, Rudd JH, Weissberg PL. Molecular and metabolic imaging of atherosclerosis. J Nucl Med 2004;45:1898-907.

32. Schaar JA, De Korte CL, Mastik F, Strijder C, Pasterkamp G, Boersma E, et al. Characterizing vulnerable plaque features by intravascular elastography. Circulation 2003;108:2636-41.

33. Cai J, Hatsukami TS, Ferguson MS, Kerwin WS, Saam T, Chu B, et al. In vivo quantitative measurement of intact fibrous cap and lipid-rich necrotic core size in atherosclerotic carotid plaque: comparison of high-resolution, contrast-enhanced magnetic resonance imaging and histology. Circulation 2005;112:3437-44.

34. Lovett JK, Gallagher PJ, Rothwell PM. Reproducibility of histological assessment of carotid plaque: implications for studies of carotid imaging. Cerebrovasc Dis 2004;18:117-23.

INVITED COMMENTARY

A. Ross Naylor, MD, FRCS, *Leicester, United Kingdom*

How many of you consider gender when deciding whether carotid endarterectomy (CEA) might be appropriate in patients with asymptomatic carotid disease? Chances are, relatively few! Yet, the Asymptomatic Carotid Atherosclerosis Study (ACAS) showed no conclusive evidence that surgery conferred benefit in women, and once perioperative strokes were included, neither did the Asymptomatic Carotid Surgery Trial (ACST). When the data from the two trials are combined, the gender differences are difficult to ignore.[1]

Two observations from the trials might explain why women gained less benefit. First, women had a lower natural history of stroke risk than men. Second, women incurred higher morbidity and mortality after CEA, a phenomenon common to many cardio-vascular operations. Accordingly, the overall benefit from CEA will be diminished. So should all asymptomatic women now be denied surgery? Definitely not!

It is, however, becoming untenable to simply treat all asymptomatic men and women as if they derived equivalent benefit. Combined ACAS and ACST data[1] indicate that CEA conferred a twofold reduction in stroke in men (odds ratio, 2.0; 95% confidence interval, 1.5 to 2.8) compared with neutral benefit in women (OR 1.04; 95% confidence interval, 0.7 to 1.6). However, because the confidence intervals straddle "1," there is still a degree of statistical uncertainty. It is inevitable, therefore, that certain women will gain considerable benefit from surgery, but can they be identified?