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ORIGINAL ARTICLE

Sex-associated differences in the modulation of vascular risk in patients with asymptomatic carotid stenosis

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In this study, we aimed to identify determinants of the different sex-related stroke risk in subjects with asymptomatic internal carotid artery (ICA) stenosis. In all, 492 women (44.4%) and 617 men (55.6%), with unilateral ⩾60% asymptomatic ICA stenosis, were prospectively evaluated with a median follow-up of 37 months (interquartile range, 26 to 43). Vascular risk profile, plaque characteristics, stenosis progression, and common carotid artery intima-media thickness were investigated. Outcome measure was the occurrence of ischemic stroke ipsilateral to ICA stenosis. Myocardial infarction, contralateral stroke and transient ischemic attack were considered as competing events. The incidence rate of ipsilateral stroke over the entire follow-up period was 0.16%: 0.09% (95% confidence interval (CI) 0.05 to 0.15) in women and 0.22% (95% CI 0.17 to 0.29) in men (log-rank test, $P < 0.001$). Stenosis progression significantly influenced the risk of ipsilateral stroke in both men (subhazard ratio, SHR, 8.99) and women (SHR 4.89). Stenosis degree (71% to 90%, SHR 2.35; 91% to 99%, SHR 3.38) and irregular plaque surface (SHR 2.32) were relevant risk factors for ipsilateral stroke only in men. Our findings suggest that characteristics of the stenosis and plaque exert a different effect in modulating vascular risk in the two sexes. Understanding sex differences in cardiovascular disease could help to target sex-specific future therapies.

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INTRODUCTION

Asymptomatic carotid stenosis is a well-documented risk factor for ischemic stroke. After initial enthusiasm, supported by clinical randomized trials, demonstrating that the surgical correction of severe carotid stenosis in primary prevention is able to positively reduce the incidence of stroke by approximately 30% over 3 years,[1] a critical reconsideration has been underway, especially after the widespread implementation of medical therapy modulating vascular risk factors. A large amount of evidence suggests that medical treatment may have benefits overcoming those expected by invasive approaches.[2–4] Accordingly, efforts have been made to improve the selection of patients whose risk profile would make them ideal candidates for invasive treatments.[5–7] Further evidence exists about a sex difference in the risk profile of patients with asymptomatic carotid stenoocclusive disease, and it has been well established that carotid endarterectomy (CEA) is more beneficial for men than for women.[8] The underlying pathophysiologic mechanism explaining this sex-specific difference is still poorly understood.[9]

In this study, we aimed to identify possible noninvasive determinants of the different sex-related stroke risk associated with asymptomatic carotid stenosis in the attempt to develop a predictive model to be applied in men and women for a more reliable and sex-specific preventive approach. For this purpose, we conducted a prospective investigation on asymptomatic subjects, integrating clinical features and ultrasonographic data concerning the characteristics of the carotid wall and plaques.

MATERIALS AND METHODS

Ethical approval was obtained from the ethics committees of Marche Polytechnic University and Campus Bio-Medico University and written informed consent was obtained in all cases in accordance with the Declaration of Helsinki.

During a 7-year period (January 2003 to December 2009), we diagnosed, by means of ultrasound according to international guidelines,[10] an internal carotid artery (ICA) stenosis of 60% or more in 1,400 asymptomatic subjects. All subjects were referred by their primary care physicians or other specialists for carotid atherosclerosis in the setting of a local primary prevention initiative. Subjects were eligible if they had not had symptoms attributable to ICA stenosis in the preceding 6 months, defined by a neurologist as the absence of transient ischemic attacks, amaurosis fugax, or stroke.

All subjects were educated on the estimated annual stroke risk ipsilateral to ICA stenosis[1] and on the advantages and disadvantages of medical versus surgical along with medical treatment, according to international guidelines.[11] Patients who considered CEA were referred to our Vascular Surgery Department and excluded from the analysis. The remaining subjects underwent neurologic and cardiac examination, including electrocardiogram, transthoracic or transesophageal echocardiography, and brain-computed tomography or magnetic resonance imaging. Exclusion criteria were previous stent implantation or CEA or coexisting severe medical conditions. We also excluded subjects with contralateral carotid stenosis ⩾60% and patients with embolizing heart diseases.

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Vascular risk factors were recorded: hypertension was defined as a systolic blood pressure of 140 mm Hg or higher and a diastolic blood pressure of 90 mm Hg or higher; diabetes was a concentration of fasting plasma glucose of 6.7 mmol/l (120 mg/dL) or more; dyslipidemia was a low-density lipoprotein cholesterol level greater than 160 mg/dL and high-density lipoprotein cholesterol level less than 35 mg/dL, or triglyceride level greater than 170 mg/dL. For smoking, we considered the current status. As regards cardiac comorbidities, we considered history of coronary artery disease (defined as documented symptomatic myocardial infarction, angina, coronary artery bypass surgery, or angioplasty). The diagnostic criteria and pharmacological treatments of vascular risk factors, in addition to behavioral recommendations (smoking cessation, regular physical exercise, weight control), were planned in accordance with international guidelines.[11,12]

Carotid arteries were assessed and defined by continuous wave Doppler and Color flow B-mode Doppler ultrasound (Philips iU22, Bothell, WA, USA). Best images were digitized and stored for central reading and interpretation. The degree of stenosis was defined, according to validated criteria,[13] in three categories: 60% to 70%, 71% to 90%, and 91% to 99% by taking into consideration the maximal stenosis with respect to the carotid lumen measured distally to plaque.[14]

For measurements of intima-media thickness (IMT), a semiautomatic software was used to improve reliability and reproducibility of measurements.[15] Due to the presence of stenosis in the ICA, we only considered IMT of the common carotid arteries.[16] The mean maximum wall thickness of the near and far wall of the common carotid arteries ipsilateral and contralateral to the stenosis was used in the analyses. The cutoff for pathologic values was set at 1.0 mm.[17] Stenotic lesions were characterized on the basis of their echogenicity and surface. The assessment of plaque echolucency was based on the modified version of the Gray–Weale classification.[18] The vessel lumen was used as a reference structure for defining echolucency, and the bright echo zone produced by the media–adventitia interface at the far wall was used as the reference structure for defining echogenicity. Plaque echolucency was graded as: (1) uniformly hyperechogenic lesion; (2) mixed forms as heterogeneous, hypoechogenic and hyperechogenic, plaques; and (3) uniformly hypoechogenic. Plaque surface was defined as (1) smooth and regular; (2) mildly irregular; and (3) ulcerated.[19]

Patients were followed up by telephone interviews every 3 months and clinically reevaluated every 6 months by a designated investigator who was unaware of the clinical data as well as of the degree and side of carotid stenosis. Particular attention was paid to check and stimulate subjects' adherence to recommended preventive measures including lifestyles and pharmacologic treatments. At the end of the follow-up period, each patient was submitted to a further clinical evaluation. The occurrence of any vascular event (transient ischemic attacks, ipsilateral or contralateral ischemic stroke, myocardial infarction) and death was recorded. In the case of events not directly observed in our hospitals, clinical records were acquired to better define the event. We paid particular attention that stroke diagnosis was confirmed by brain computed tomography or magnetic resonance imaging.

During the first 12-month period, each patient was submitted to a further ultrasonographic evaluation of neck vessels to measure the possible progression of ICA stenosis. Median time for this evaluation was 8.5 months (range, 6 to 12 months) from inclusion. Stenosis progression was defined as an increase in the degree of stenosis by at least one category. All ultrasonographic examinations, including those performed to assess the progression of stenosis were performed by the same two experienced operators, masked to clinical information. To increase the reliability of the diagnosis of stenosis progression, the operators were not allowed to check the outcome of the baseline examination. Interreader reproducibility was assessed by having the two sonographers blindly selected and redigitized a B-mode carotid image from the 20-second videotape recording of a randomly selected set of 400 studies originally read by a third sonographer. Each sonographer then performed all measurements. One sonographer reread 330 studies for an interreader correlation coefficient of 0.88, and the second sonographer reread 392 studies for an interreader correlation coefficient of 0.86. Intrareader variability was also assessed by having the two sonographers reread 270 studies randomly selected and resulted 0.92 and 0.94, respectively.

Statistical Analysis

Data were analyzed using Stata/IC 11.1 (Stata, TX, USA). The following variables were evaluated: age, sex, smoking, hypertension, diabetes, dyslipidemia, coronary artery disease, pharmacological treatment of the cardiovascular risk factors, degree of carotid stenosis, Carotid IMT (normal, pathologic), plaque echolucency, plaque surface, and stenosis progression (present, absent). *t*-test and Pearson χ^2 were used to compare basal characteristics between women and men. Comparison of the detection of progressive ICA stenosis according to the sex of the subjects was performed by Pearson χ^2.

A competing-risk model was built considering only ipsilateral ischemic stroke as the first adverse outcome and all other vascular events as competing events to examine the association between sex and occurrence of ipsilateral stroke. The model was performed first in the overall cohort. The analysis was then stratified according to sex. Results of these models are presented as subhazard ratio (SHR) and 95% confidence interval (CI). If a patient experienced multiple events during follow-up, then only the first event was considered as the outcome event.

RESULTS

A total of 1,400 subjects were considered in this study. Of these, 291 were excluded for the following reasons: previous ipsilateral carotid revascularization (11); coexisting severe medical conditions or death preventing follow-up completion (22); contralateral carotid stenosis $\geqslant 60\%$ (31); embolizing cardiopathies (10); decision to be referred to the Vascular Surgery Department to perform CEA (217). Overall, 1,109 asymptomatic subjects with ICA stenosis were included in the analysis: 492 women (44.4%) and 617 men (55.6%). A preliminary analysis was performed to compare these subjects with the 217 ones excluded from the study because of their decision to be referred to the Vascular Surgery Department. No differences in demographic characteristics, vascular risk profile, and ultrasound findings emerged.

In all, 621 of the 1,109 included subjects had been already enrolled in our previous study exploring the relationship between plaque characteristics and stenosis and the risk of vascular events.[7] Demographic and clinical data are presented in Table 1.

At the first ultrasound evaluation, ICA stenosis of 60% to 70% was found in 902 subjects (81.3%), 71% to 90% in 173 subjects (15.6%), and 91% to 99% in 34 subjects (3.1%). No difference in the distribution of stenosis categories, IMT values, and plaque surface was present between men and women. However, the two groups presented a different distribution of plaque echogenicity: men had a higher prevalence of uniformly echogenic and mixed echogenicity lesions than women, while in women, the prevalence of uniformly anechogenic plaques was significantly higher ($P < 0.001$) (Table 1).

At the second evaluation, progression of ICA stenosis was detected in 231 subjects (20.8%), with no significant difference between men (141, 22.9%) and women (90, 18.3%).

No subject had a clinical event before the second ultrasound evaluation. Clinical events occurred in 145 subjects (13.1%): 96 men (15.6%) and 49 women (9.9%) ($P = 0.006$, Pearson χ^2). Of these events, 70 were strokes (48%), 62 ipsilateral and 8 contralateral to the carotid stenosis, 37 transient ischemic attack (26%) and 38 myocardial infarctions (26%).

The median follow-up period was 37 months (interquartile range, 26 to 43). The incidence rate of the composite events over this follow-up period was 0.38%: 0.29% (95% CI 0.22 to 0.38) in women and 0.45% (95% CI 0.37 to 0.55) in men. The incidence rate of ipsilateral stroke over the entire follow-up period was 0.16%: 0.09% (95% CI 0.05 to 0.15) in women and 0.22% (95% CI 0.17 to 0.29) in men (log-rank test, $P < 0.001$).

In the competing-risk regression analysis, male sex was associated with a higher risk of ipsilateral stroke (SHR 2.28, 95% CI 1.24 to 4.17, $P = 0.008$), after adjusting the model for stenosis progression (SHR 7.49, CI 3.98 to 14.11, $P < 0.001$), plaque surface

Table 1. Baseline demographic and clinical characteristics, percentage of stenosis, plaque characteristics, and IMT of the included patients

Characteristics	*Men (n = 617)*	*Women (n = 492)*	*Significance of difference (test)*
Age at examination, years (mean ± s.d.)	71.6 ± 8.6	73.5 ± 8.1	$P < 0.001$[a]
Hypertension, *n* (%)	411 (66.6)	342 (69.5)	$P = 0.304$[b]
Diabetes, *n* (%)	135 (21.9)	144 (29.3)	$P = 0.010$[b]
Dyslidipemia, *n* (%)	330 (53.5)	295 (59.9)	$P = 0.031$[b]
Smoking, *n* (%)	149 (24.2)	132 (26.8)	$P = 0.308$[b]
Coronary artery disease, *n* (%)	139 (22.5)	96 (19.5)	$P = 0.222$[b]
Use of antihypertensives, *n* (%)	384 (62.2)	326 (64.2)	$P = 0.495$[b]
Use of antidiabetics, *n* (%)	131 (21.2)	142 (28.9)	$P = 0.003$[b]
Use of statins, *n* (%)	266 (43.1)	218 (44.3)	$P = 0.690$[b]
Use of antiplatelets, *n* (%)	325 (52.7)	267 (54.3)	$P = 0.597$[b]
Stenosis category, n *(%)*			
60–70%	488 (79.1)	414 (84.2)	$P = 0.095$[b]
71–90%	107 (17.3)	66 (13.4)	
91–99%	22 (3.6)	12 (2.4)	
IMT, mm (mean ± s.d.)	1.08 ± 0.72	1.08 ± 0.76	$P = 0.984$[a]
Plaque echogenicity, n *(%)*			
Uniformly echogenic	105 (17.0)	50 (10.2)	$P < 0.001$[b]
Mixed	163 (26.4)	104 (21.1)	
Uniformly anechogenic	349 (56.6)	338 (68.7)	
Plaque surface, n *(%)*			
Regular	476 (77.2)	395 (80.3)	$P = 0.428$[b]
Irregular	114 (18.5)	80 (16.3)	
Ulcerated	27 (4.4)	17 (3.5)	

IMT, intima-media thickness. [a]Two-sample *t* test. [b]Pearson χ^2 test.

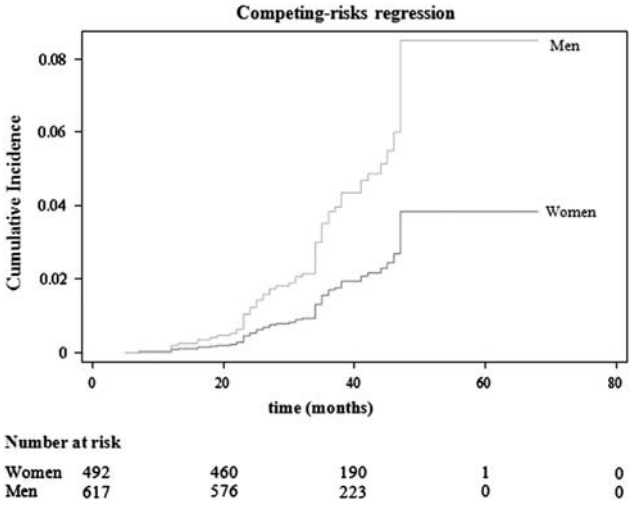

Figure 1. Competing-risk regression analysis considering ipsilateral stroke as the event of interest and all the other vascular accidents as competing events, in men and women. Male sex was associated with a higher risk of ipsilateral stroke (subhazard ratio (SHR) 2.28, 95% confidence interval (CI) 1.24 to 4.17, $P = 0.008$), after adjusting the model for stenosis progression, plaque surface, and degree of stenosis.

(irregular, SHR 1.91, 95% CI 1.04 to 3.51, $P = 0.037$; ulcerated, SHR 2.05, 95% CI 0.91 to 4.63, $P = 0.083$), and degree of stenosis (71% to 90%, SHR 2.41, 95% CI 1.34 to 4.34, $P = 0.003$; 91% to 99%, HR 3.94, 95% CI 1.54 to 10.07, $P = 0.004$) (Figure 1). When the same analysis was stratified by sex, stenosis progression was found to be the only significant risk factor shared by both sexes, although its predictive value was higher in men than in women (SHR 8.99 versus 4.89). Degree of stenosis (71% to 90%, SHR 2.35; 91% to 99%, SHR 3.38) and irregular plaque surface (SHR 2.32) resulted as relevant risk factors for ipsilateral stroke only in men (Table 2).

Table 2. Competing-risk regression models considering ipsilateral stroke as the event of interest and all the other vascular accidents as competing events in women (W) and men (M)

Variables	*Subhazard ratio*		*P-value*		*95% Confidence interval*	
	W	*M*	*W*	*M*	*W*	*M*
Plaque progression	4.89	8.99	0.007	< 0.001	1.55–15.52	4.24–19.06
Stenosis degree						
60–70%	Ref	Ref	–	–	–	–
71–90%	2.93	2.35	0.070	0.018	0.92–9.33	1.16–4.77
91–99%	6.02	3.38	0.083	0.016	0.79–45.81	1.25–9.15
Plaque surface						
Regular	Ref	Ref	–	–	–	–
Irregular	0.90	2.32	0.894	0.013	0.19–4.09	1.19–4.51
Ulcerated	3.04	1.67	0.155	0.289	0.66–14.06	0.65–4.32

DISCUSSION

The potential effect of sex stratification in the outcome of patients with ICA stenosis may have important implications in clinical practice. International guidelines suggest that sex should be taken into account when selecting a treatment option, in particular a revascularization procedure for an individual patient.[20,21]

Our findings confirm that outcomes in vascular disease differ between sexes. We examined the risk related to the presence of an asymptomatic carotid stenosis $\geqslant 60\%$ since in this group of subjects controversies about the need of the most aggressive treatments, including CEA are still present.[1] However, we excluded patients with lower degrees of stenosis in which the risk of ipsilateral stroke is not significantly increased.

According to previous results,[22] in our population, men had a higher risk of stroke ipsilateral to the stenosis with respect to women. This different risk profile was not reliably explained by considering the baseline characteristics. In fact, the distribution of most vascular risk factors and stenosis/plaque characteristics was not able to differentiate men from women. Indeed, a higher mean age and a higher prevalence of diabetes and dyslipidemia appeared to disadvantage women in terms of vascular risk extent. These data further underline the possibility that the risk of stroke ipsilateral to carotid stenosis is modulated by complex interactions between vascular factors and sex-related conditions. Accordingly, the relevant aspect of our findings is that some plaque characteristics seem to exert a different effect in modulating the vascular risk in the two sexes. In particular, irregular plaque surface and a $>70\%$ degree of stenosis were able to identify men with the highest risk of ipsilateral stroke. The progression of stenosis was the only characteristic able to identify an increased stroke risk in both sexes, even if its predictive value was significantly higher in men than in women. This relevant role of stenosis progression in characterizing the predisposition to develop ipsilateral stroke has been documented in previous studies in which, however, the sex-related differences in risk profile were not specifically evaluated.[23,24] In our study, the percentage of subjects who showed a 1-year progression in the extent of carotid stenosis was

quite relevant (20.8%). This progression rate was probably influenced by the particular characteristics of our study sample. Patients were recruited in the setting of a preventive initiative mainly devoted to subjects with an aggregation of vascular risk factors. Further, an optimization of treatments for vascular risk factors was obtained at the moment of the inclusion in the study. It is possible that within the first year of observation, a satisfactory effect on the reduction of the risk of stenosis progression had not yet obtained. Another aspect requiring a comment is the lack of an apparent effect of plaque anechogenicity, that we found more frequently present in women, in modulating the risk of ipsilateral stroke. A possible explanation is that a uniform anechogenicity results in a lower level of risk than the presence of a mixed echogenicity. This hypothesis seems also to be supported by the results of recent studies investigating the possibility of a cerebrovascular risk stratification on the basis of ultrasonic plaque characteristics in patients with asymptomatic ICA stenosis.[7,25] The lack of a significant effect of irregular plaque surface and degree of stenosis in the modulation of stroke risk in women is in accordance with the findings of a previous prospective cohort study whose results suggested the possibility that a lower plaque burden for the same type of ICA stenosis may characterize females with respect to males.[26] The difference may be based on a different and sex-specific capability of arterial remodeling. The underlying pathophysiologic mechanisms explaining the sex specific differences in the vascular risk are poorly understood. According to our data, more than just a difference in the clinical and demographic aspects and in the pathophysiologic characteristics of the plaque, the modulation of the vascular risk seems to be based on a peculiar different interaction among them and patients' sex. The influence of intrinsic factors, such as the sex steroids hormones, coagulation, and the genetics, which can be modulated by epigenetic mechanisms over the lifetime, has been hypothesized to explain the sex-related differences in vascular diseases. Previous studies have postulated a role of the higher estrogen status in women in determining a cerebrovascular protection.[27,28] In experimental models of stroke induced by middle cerebral artery occlusion, females were more protected against ischemic damage than males.[29] This effect was not confirmed in ovariectomized females, but it was rescued by estrogen supplementation, thus supporting the neuroprotective function of estrogens.[30] In our investigation, the possibility of a risk modulation induced by estrogens cannot be established with certainty since our population was elderly, and the number of women with hormonal replacement therapy was very low (about 8%). However, it is possible that a protective hormonal effect remains lifelong in women, even if to a reduced level. Sex differences in platelet biology and coagulation reactions that can influence the effects of antithrombotic therapy have been described.[31] Regarding a potential genetic influence, the sex chromosomes themselves have been identified as possible candidates for influencing sex differences in the clinical expression of cerebrovascular diseases. A sexually different response to ischemia has been shown in animal models,[32] showing also a corresponding difference in the expression levels of a specific set of mitochondrial RNAs.[33]

Our data may not add a pathophysiologic explanation for the different vascular risk between men and women with asymptomatic ICA stenosis, but they could be interpreted as a preliminary evidence in the attempt to build an individual risk profile for patients according to sex. The study was performed in a controlled setting with a careful attention to treatment adherence for the management of vascular risk factors as part of an initiative for primary prevention in the context of a program of health surveillance. Accordingly, the large majority of subjects remained free from vascular events during our observation period. This further underlines the need to find reliable markers that are able to select the subgroup of subjects, even if of limited size, in which a standard pharmacological approach cannot be considered sufficient to completely exclude the risk of vascular events. In this respect, when considering baseline clinical aspects and the characteristics of the plaque and of the stenosis, attention should be paid to patients' sex for the possibility of its impact in modulating vascular risk. From a practical point of view, our results, obtained in a population of asymptomatic subjects with ICA stenosis $>60\%$, suggest that in men a higher degree of carotid stenosis, an irregular plaque surface and a progression of stenosis can identify subjects at increased risk of ipsilateral stroke. In women, this possibility is limited to the individuation of subjects in which the stenosis shows a progression over a 12-month period.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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