# Serum values of metalloproteinase-2 and metalloproteinase-9 as related to unstable plaque and inflammatory cells in patients with greater than 70% carotid artery stenosis

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***Objective:*** **Unstable carotid plaques, characterized by increased levels of macrophages and T lymphocytes, have high emboligenic potential and carry a risk for producing cerebral ischemic events. It has been suggested that plaque instability may be mediated by the family of metalloproteinases (MMPs). The purpose of this study was to analyze the relationship between concentrations of MMP-2 and MMP-9 and unstable carotid plaques, presence of macrophages and T-lymphocytes in the plaques, and neurologic symptoms, to establish additional risk markers in patients with greater than 70% carotid artery stenosis. This was a cross-sectional study carried out in a referral center and institutional practice in hospitalized patients.**

***Methods:*** **The study included 40 patients with carotid artery stenosis treated with carotid endarterectomy. Of these patients, 67.5% had experienced a previous neurologic event and 32.5% exhibited no symptoms. MMP-2 and MMP-9 levels were determined with enzyme-linked immunosorbent assay 48 hours before surgery. Histopathologic analysis (stable or unstable) and immunohistochemistry (macrophage count, T lymphocytes, activated T lymphocytes) were carried out on the plaques.**

***Results:*** **Mean MMP-2 and MMP-9 serum concentrations in the population studied were 1138.27 ± 326.08 ng/mL and 1026.10 ± 412.90 ng/mL, respectively. MMP-2 levels were significantly higher in patients with symptoms compared with patients without symptoms (1247.30 ± 276.80 ng/mL vs 911.80 ± 311.84 ng/mL; $P = .001$). MMP-9 was also significantly higher in the symptomatic group (1026.10 ± 412.90 ng/mL vs 377.84 ± 164.08 ng/mL; $P = .001$) and in patients with unstable plaques compared with those with stable plaques (1006.98 ± 447.09 ng/mL vs 496.16 ± 292.78 ng/mL; $P = .001$). In addition, we found a strong association between elevated MMP-9 concentration and presence of macrophages in plaque (Spearman rho, 0.45; $P = .004$). At logistic regression analysis, variables that best predicted the presence of unstable plaque were a previous neurologic event and MMP-9 level greater than 607 ng/mL (sensitivity, 96%; specificity, 92%; negative predictive value, 94.7%; positive predictive value, 93%).**

***Conclusion:*** **Elevated MMP-9 concentration is associated with carotid plaque instability and the presence of macrophages, factors that indicate increased risk for a neurological event. Determination of this gelatinase may enable identification of high-risk subgroups of patients with carotid artery stenosis. (J Vasc Surg 2004;40:469-75.)**

Formation of atherosclerotic plaque is a dynamic process that involves various phenomena, such as macrophage and lymphocyte migration, proliferation of smooth muscle cells, neovascularization, and repair and remodeling of the extracellular matrix. Recent studies have suggested that the different phases of atherosclerosis may be mediated by the family of metalloproteinases (MMPs),[1,2] zinc-dependent physiologic regulators of the extracellular matrix. Although it is not completely certain how MMP production is induced by the extracellular matrix, these gelatinases are necessary for infiltration of monocytes and T lymphocytes to occur in the subendothelial spaces. Activated endothelial cells express adhesion molecules, such as vascular cell adhesion molecule–1, which promote infiltration of circulating monocytes and T lymphocytes. Adhesion of these cells to the endothelial cells could induce production of MMP-2 (72 kD, gelatinase A), which facilitates breakdown of the extracellular matrix.[3] Furthermore, contact with type I collagen and laminin in the matrix increases expression of MMP-9 (92 kD, gelatinase B), and interleukin-1 secretion can activate pro-MMP-2 and pro-MMP-9 produced by smooth muscle cells, which could lead to endothelial disruption or intraplaque hemorrhage.

There is evidence[4,5] that unstable carotid plaques, that is, those with ulceration or recent intraplaque hemorrhage, are significantly more prevalent in patients with a history of ischemic neurologic symptoms due to microembolism secondary to plaque dislodgment, and it seems feasible that MMPs could be implicated in the process, leading to plaque instability.

The purpose of this study was to determine the levels of MMP-2 and MMP-9 in a population of candidates for carotid surgery with greater than 70% carotid artery steno-

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sis, and to correlate the levels of these MMPs with a history of neurologic symptoms, carotid plaque anatomy (stable or unstable), and carotid plaque cell types related to inflammation (macrophages, T lymphocytes, activated T lymphocytes).

Determination of systemic biologic parameters that indicate the presence of unstable plaques could be a useful diagnostic indicator for selection of high-risk cohorts in patients with stenosis of the extracranial internal carotid artery.

## PATIENTS AND METHODS

**Patients.** Between September 2001 and April 2002, 52 patients with greater than 70% carotid artery stenosis were admitted to our center for carotid thromboendarterectomy. None of these patients had gastric, hepatic, or colonic neoplasms, rheumatoid arthritis, or comorbid conditions that can increase MMP expression. Local ethical committee approval was obtained for the procurement of specimens, and all patients gave full informed consent for participation in the study. The protocol we used for later data analysis was as follows: collection of clinical and paraclinical data, blood sample collection for MMP quantification, performance of carotid surgery, and preservation of carotid plaques for histologic analysis and immunohistochemistry. In 7 patients plaque processing was inadequate, and in 5 patients blood sample volume was insufficient for MMP determination. A final total of 40 patients were included in the study.

The study patients were 34 men and 6 women, with ages ranging from 48 to 83 years (mean, 65.1 ± 10.1 years). Twenty-seven patients had experienced a previous neurologic event, and 13 patients had no symptoms. In addition to standard epidemiologic variables (age, sex), the following classic cardiovascular risk factors were recorded: hypertension, defined as systolic blood pressure 140 mm Hg or greater or diastolic blood pressure 90 mm Hg or greater, or current use of antihypertensive medication; diabetes mellitus, defined as a glycosylated hemoglobin $A_{1c}$ concentration greater than 5.8% or current use of hypoglycemic agents; hypercholesterolemia, defined as a total cholesterol concentration 220 mg/dL or greater or current use of cholesterol-lowering agents; and smoker, defined as current smoking or cessation of smoking less than 1 month before entering the study. Furthermore, we investigated the presence of concomitant cardiovascular disease, including peripheral vascular disease and ischemic heart disease, treatment with antithrombotic agents and lipid-lowering drugs, and interval between the neurologic event and blood collection. We followed the North American Symptomatic Carotid Endarterectomy Trial criteria[6] to classify the patients as neurologically symptomatic or asymptomatic for the study. Degree of carotid stenosis was determined with Doppler ultrasound scanning (Philips SD800) of the supraaortic trunks, and with brain angiography in selected patients,[7] including those with greater than 70% stenosis on one side and greater than 50% stenosis on the other side, with contralateral occlusion or with inconclusive findings on the Doppler ultrasound scan. The brain parenchyma was studied with computed tomography in the first cases included in the protocol (n = 32), and with magnetic resonance imaging in later cases (n = 8).

**MMP-9 and MMP-2 determinations.** Twenty-four hours before surgery, peripheral blood samples were collected in tubes containing ethylenediamine tetraacetic acid (EDTA). After centrifugation the serum was siphoned into freezing vials, snap-frozen in liquid nitrogen, and stored at −80°C. MMP-2 and MMP-9 levels were determined with a commercially available enzyme-linked immunosorbent assay (ELISA), using Biotrak assay systems (Amersham) for MMP-2 and Quantikine (R&D Systems) for MMP-9, validated for use with human serum. ELISAs were performed according to the manufacturers' instructions. Normal reference values, provided by the manufacturers and validated by our laboratory in samples from 37 healthy volunteers, for MMP-2 were mean, 633 ng/mL (range, 470-800 ng/mL), and for MMP-9 were mean, 436 ng/mL (range, 169-705 ng/mL). Mean intra-assay coefficients of variation for the method were 2.9% for MMP-9 and 5.4% for MMP-2.

**Histopathologic analysis.** Carotid endarterectomy was performed in a conventional series of patients with conventional surgical techniques. In brief, the carotid artery was incised, and the plaque was removed from within the lumen as a single specimen. For histopathologic analysis the surgical specimen was fixed in 10% formalin, decalcified with EDTA (Surgipath Medical Industries) over 4 hours, cut transversally in 2-mm blocks, processed, and embedded in paraffin. The blocks were sectioned at 4 μm, and were stained with hematoxylin-eosin, van Gieson stain for elastic fibers, and Masson trichrome stain. After the specimens were prepared for histologic analysis they were examined with an optic microscope (Dialux 20EB; Leitz) by a pathologist blinded to MMP levels and to the presence or absence of neurologic symptoms.

Morphologic characteristics of the carotid plaques were established according to the classification of the American Heart Association.[8] All plaques were graded as type V or VI (advanced lesions) with this classification, defined as follows: type V, fibrous or stable, characterized by fibrous conjunctive tissue together with extracellular lipids and laminated acellular collagen, with no evidence of endothelial disruption; and type VI, complicated or unstable, characterized by ulceration (disruption of the endothelial surface) or recent intraplaque hemorrhage consisting of diffuse blood (polymorphonuclear infiltrate accompanying intact red blood cells) in the subendothelial space.

**Immunohistochemistry.** For immunohistochemistry of the tissue, the thin paraffin-embedded sections were deparaffinized in xylene, rehydrated through graded alcohols, and preincubated in 10% hydrogen peroxide for 10 minutes. Antigen unmasking was performed at high temperature and pressure. The samples were then incubated with specific antibodies for macrophages, T lymphocytes, and activated T lymphocytes for 60 minutes at room tem-

**Table I.** Clinical and epidemiologic characteristics of study population

| | *Symptomatic (n = 27)* | | *Asymptomatic (n = 13)* | | |
|---|---|---|---|---|---|
| | *n* | *%* | *n* | *%* | P |
| Age (y; mean ± SD) | 65.5 ± 11 | | 64.4 ± 8.2 | | NS |
| Male gender | 23 | 85.1 | 11 | 84.6 | NS |
| Body mass index | | | | | |
| Mean | 24 | | 23 | | NS |
| Range | 19–39 | | 1–28 | | |
| Hypertension | 11 | 40.7 | 7 | 53.8 | NS |
| Smoker | 18 | 66.6 | 9 | 69.2 | NS |
| Hypercholesterolemia | 13 | 48.1 | 4 | 30.7 | NS |
| Diabetes mellitus | 11 | 40.7 | 6 | 46.1 | NS |
| Peripheral vascular disease | 16 | 59.2 | 8 | 61.5 | NS |
| Ischemic heart disease | 10 | 37 | 7 | 53.8 | NS |
| Antithrombotic agents | 11 | 40.7 | 8 | 61.5 | NS |
| Lipid-lowering drugs | 10 | 37 | 6 | 61.5 | NS |

*NS*, Not significant.

perature. The primary antibodies (DAKO) were monoclonal for the macrophages (CD68) and activated T lymphocytes (HLA-DR), and polyclonal for the T lymphocytes (CD3). Subsequently, samples were incubated with biotin-labeled secondary antibodies (Dako Duet; DAKO). Diaminobenzidine was used for development. Finally, the blocks were stained with hematoxylin-eosin for contrast, and were examined with an optic microscope. Differential cell count was carried out with a standard morphometry method: random choice of 10 fields for each preparation, and cell count for each type of cell in each field. Results are expressed as number of cells per 10 fields.

**Statistical analysis.** Statistical analysis of the data was performed with the SPSS for Windows, version 10.0. Categorical variables are described in frequencies, because they followed a normal distribution, with the mean as the central measurement and the standard deviation as the measure of dispersion. Variables with a non-normal distribution are expressed in median and range. Associations were considered statistically significant at $P < .05$. The $\chi^2$ test was used to establish associations among categorical variables, and the Mann-Whitney *U* nonparametric test for variables that did not follow a normal distribution. Comparisons between quantitative variables were performed with the Spearman correlation coefficient. A receiver operator characteristic curve was used to establish the value for macrophage number associated with unstable plaque. Multivariate analysis was carried out by creating a logistic regression model with the forward stepwise method.

## RESULTS

The prevalence of epidemiologic variables in neurologically symptomatic and asymptomatic patients is represented in Table I. In the symptomatic group (n = 27), 17 patients (62.9%) had a transient ischemic attack (7 (41.1%) retinal, 10 (58.8%) hemispheric) and 10 patients (37%) had stroke. Doppler ultrasound scanning of the supra-aortic trunk was performed in all patients to establish the degree of stenosis, and in 15 patients (37.5%) cerebral angiography was also indicated. To study the brain parenchyma, computed tomography was performed in 32 patients (80%) and MRI in 8 patients (20%). Of these studies, 57.5% were interpreted as normal, 12.5% of patients exhibited cortical atrophy, and 30 patients had ischemic areas corresponding to the region of study. No clinically silent infarctions were detected.

At histologic analysis 62.5% of plaques were classified as unstable and 37.5% as stable. Regarding differential cell count, in both types of plaques the most frequently encountered cells were macrophages, followed by T lymphocytes and activated T lymphocytes. A median of 17 macrophages per 10 fields (range, 2-45) was found in unstable plaques, and 5 macrophages per 10 fields (range, 2-10) in stable plaques. T lymphocyte count was 3 cells per 10 fields (range, 0-7) in unstable plaques, and 1 cell per 10 fields (range, 0-5) in stable plaques. Activated T lymphocytes presented a median of 2 cells per 10 fields (range, 0-5) in unstable plaques, and 0 cells per 10 fields (range, 0-2) in stable plaques. Differences between the 2 groups were significant ($P < .001$). Unstable plaques were significantly associated with the presence of neurologic symptoms ($P < .01$).

**MMP-2 neurologic symptoms, plaque types, and cells.** Mean (± SD) MMP-2 concentration in the total series was 1138.27 ± 326.08 ng/mL, with significant differences as compared with the reference value ($P < .001$). MMP-2 was higher (1247.30 ± 276.80 ng/mL) in patients with a previous neurologic event than in patients with no symptoms (911.80 ± 311.84 ng/mL) ($P<0.001$). There were no differences in MMP-2 levels in the analyses performed within 1 month of the neurologic event and those performed later. Mean MMP-2 level was 1209.47 ± 244.38 ng/mL in patients with plaques considered unstable, and 1019.60 ± 411.91 ng/mL in patients with stable plaques ($P = .07$; Fig 1). There was no association between

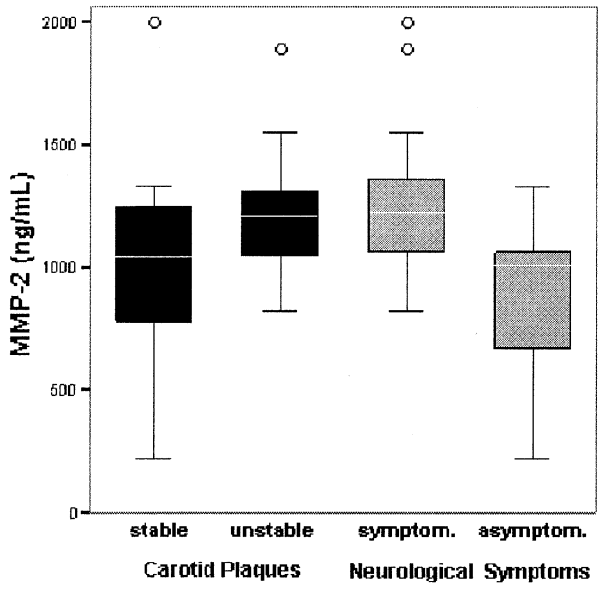


**Fig 1.** MMP-2 concentration relative to plaque structure (stable or unstable) and neurologic events.

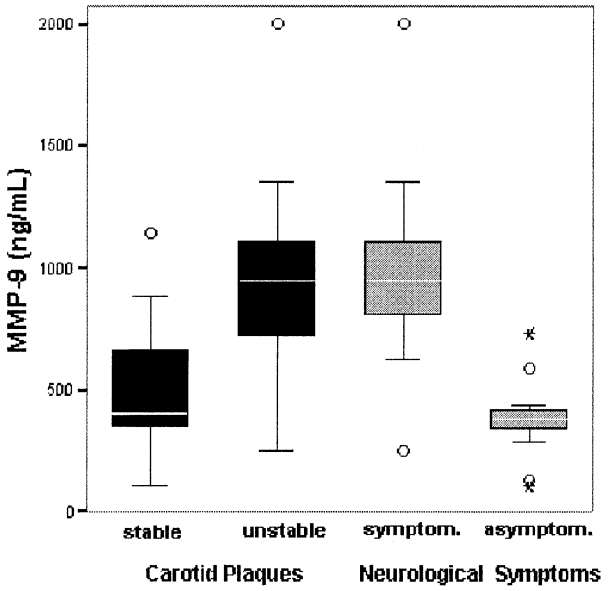


**Fig 2.** MMP-9 concentration relative to plaque structure (stable or unstable) and neurologic events.

increased MMP-2 concentration and any of the cell types identified at immunohistochemistry.

**MMP-9 neurologic symptoms, plaque types, and cells.** MMP-9 concentration in the entire study cohort was 1026.10 ± 412.90 ng/mL, with significant differences as compared with the reference value ($P < .001$). There was a clear association between increased MMP-9 concentration and a previous neurologic event. Mean MMP-9 values in

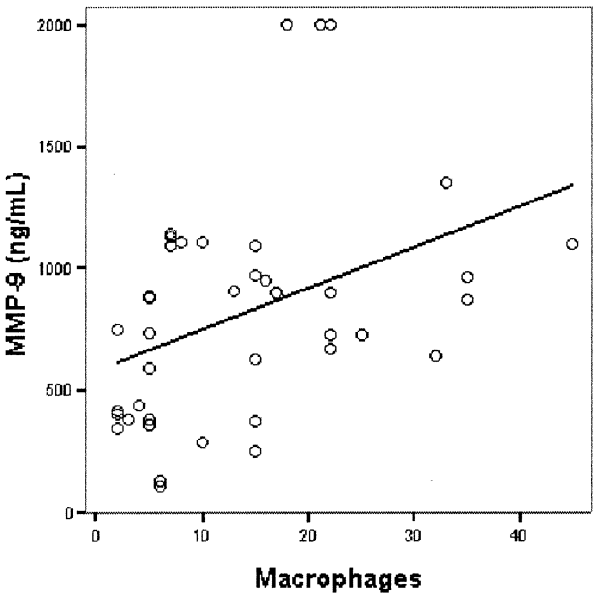


**Fig 3.** MMP-9 concentration relative to number of macrophages in plaque.

patients with symptomatic disease was 1026.10 ± 412.90 ng/mL, versus 377.84 ± 164.08 ng/mL in patients with asymptomatic disease ($P < .001$). In patients in whom MMPs were determined and plaques were examined within the first month after the neurologic event, MMP-9 levels were significantly higher than in patients examined after the first month: 1213.4 ± 390.4 ng/mL versus 832.55 ± 389.1 ng/mL ($P = .01$). We found a strong association between unstable plaque and high MMP-9 levels: 1006.98 ± 447.09 ng/mL in patients with unstable plaques and 496.16 ± 292.78 ng/mL in patients with stable plaques ($P < .001$; Fig 2).

Regarding cell types, we found that the greater the number of macrophages in the plaques the higher the levels of MMP-9 (Spearman rho 0.45; $P = .004$; Fig 3). There were no differences in number of macrophages or lymphocytes between plaques analyzed within 1 month after the event and those analyzed later.

There were no significant differences between the various cardiovascular risk factors and MMP overexpression or between drug use (antithrombotic or lipid-lowering agents) and MMP levels (Table II).

The cutoff point for MMP-9 that best predicted the presence of unstable plaque was 607 ng/mL (odds ratio [OR], 19.20; 95% confidence interval [CI], 3.91-94.18; $P < .001$), with sensitivity 96%, specificity 92%, positive predictive value 93%, and negative predictive value 94.7%. After logistic regression the presence of neurologic symptoms was the only variable that remained in the model as an independent predictor of unstable plaque (OR, 40.625; 95% CI, 6.55-251.96; $P < .001$; Table III).

**Table II.** Association between cardiovascular risk factors, neurologic events, plaque structure, cell type, and MMP-2 and MMP-9 concentrations

| | *Unstable (n = 25)* | | *Stable (n = 15)* | | |
|---|---|---|---|---|---|
| | *n* | *%* | *n* | *%* | P |
| Age (y; mean ± SD) | 65.8 ± 11 | | 64.4 ± 8.5 | | NS |
| Male gender | 23 | 85.1 | 11 | 84.6 | NS |
| Body mass index | | | | | |
| Mean | 24 | | 23 | | NS |
| Range | 19–39 | | 1–28 | | |
| Hypertension | 9 | 36 | 9 | 60 | NS |
| Smoker | 16 | 64 | 11 | 73.3 | NS |
| Hypercholesterolemia | 13 | 52 | 4 | 26.7 | NS |
| Diabetes mellitus | 10 | 40 | 7 | 46.7 | NS |
| Peripheral vascular disease | 14 | 56 | 10 | 66.7 | NS |
| Ischemic heart disease | 10 | 40 | 7 | 46.7 | NS |
| Antithrombotic agents | 10 | 40 | 9 | 60 | NS |
| Lipid-lowering drugs | 9 | 36 | 7 | 46.7 | NS |
| Neurologic event | 24 | 96 | 3 | 20 | <.001 |
| Lesion CT or MRI | 9 | 36 | 3 | 20 | NS |
| MMP-2 (mean ± SD) | 1209.5 ± 244.4 | | 1019.6 ± 411.9 | | NS |
| MMP-9 (mean ± SD) | 1007.0 ± 447.1 | | 496.2 ± 292.8 | | <.001 |
| Macrophages | | | | | |
| Median | 17 | | 5 | | <0.001 |
| Range | 2–45 | | 2–10 | | |
| T lymphocytes | | | | | |
| Median | 3 | | 1 | | .001 |
| Range | 0–7 | | 0–5 | | |

*MMP*, Matrix metalloproteinase; *CT*, computed tomography; *MRI*, magnetic resonance imaging; *NS*, not significant.

## DISCUSSION

The results of this study uphold the hypothesis that MMPs are implicated in the mechanisms that cause destabilization of atherosclerotic plaque and in the pathogenesis of cerebral ischemic events, through interactions with immune system cells, macrophages, and T lymphocytes. Several studies have related elevated levels of these gelatinases with atherosclerotic plaque instability. Kai et al[9] determined serum MMP-2 and plasma MMP-9 concentrations in 33 patients with acute coronary syndrome and in 17 healthy control subjects, and found that patients with unstable angina or acute myocardial infarction had MMP-2 levels up to twice as high and MMP-9 levels 2-fold or 3-fold higher than those in healthy control subjects. Along this same line, Inoue et al[10] reported elevated MMP-1 and MMP-3 concentrations in patients with unstable angina or acute myocardial infarction compared with healthy subjects or patients with stable exertional angina. More recently, Morgan et al[11] studied levels of MMP-1, MMP-3, MMP-7, MMP-9, and MMP-12 in relation to carotid plaque morphologic characteristics and neurologic symptoms, and concluded that MMP-1 and MMP-12 determine atherosclerotic plaque stability. With regard to the mechanisms implicated in the production of MMPs, in addition to monocytes and T lymphocytes some authors have demonstrated platelet involvement. Fernandez-Patron et al[12] identified MMP-2 and MMP-9 in human platelets, and suggested that the MMP-2 or MMP-9 system may have an important role in regulation of platelet-platelet and platelet-vessel wall interactions. Furthermore, in a study examining the cellular interactions occurring in plaque destabilization, Galt et al[13] found that monocyte interaction with collagen and platelets is required for these leukocytes to synthesize MMP-9 and that the interactions are produced particularly in areas of the vessel where inflammatory phenomena develop in response to injury.

**Table III.** Factors associated with unstable plaque in logistic regression model

| | *OR* | *95% CI* | P |
|---|---|---|---|
| | | | <.001 |
| Clinical | 40.625 | 6.55–251.96 | |
| MMP-9 | 19.20 | 3.91–94.18 | <.001 |

*MMP*, Matrix metalloproteinase; *OR*, odds ratio; *CI*, confidence interval.

We found significantly higher MMP-9 concentrations in patients with previous neurologic symptoms and unstable plaques as determined at histologic analysis, and a strong association between MMP-9 overexpression and the presence of macrophages in the plaques. In contrast, MMP-2 concentrations were only slightly higher in the symptomatic group than in the asymptomatic group, but there was no association with any of the cell types studied.

The increased MMP-9 concentration in these patients could result from extracellular matrix breakdown and repair activity in response to endothelial rupture or interplaque hemorrhage (in response to plaque instability), or it could be produced with cerebral ischemia. However, analysis of ischemic lesions in the brain parenchyma and MMP-9

concentration disclosed ischemic areas corresponding to the region of study in 30% of patients, and unstable plaques in 62.5% of patients, with associations between MMP-9 overexpression and presence of unstable plaques but not areas of cerebral ischemia. Moreover, the interval between cerebral infarction and MMP determination in the present study was 6.54 ± 3.44 weeks. In patients with MMP determinations and plaque analysis within the first 30 days after the ischemic event, MMP-9 levels were significantly higher than in those studied after 30 days. With regard to the presence of macrophages and lymphocytes, there were no such time-related differences.

This leads us to believe that the increased levels of this gelatinase were due to plaque instability and not to cerebral injury. Along this line, works such as those by Loftus et al[14,15] have demonstrated that there is a local increase in active MMP-9 concentration in most unstable carotid plaques when these are defined by the presence of focal neurologic symptoms and cerebral microembolism detected at transcranial Doppler scanning. Recently Molloy et al[16] studied MMP-9 levels in patients undergoing carotid endarterectomy in whom microembolisms were detected with transcranial Doppler scanning during surgery. The authors reported that patients with microembolisms had higher MMP-9 levels 48 hours later, and attributed this elevation to the cerebral injury.

Some interesting work has been done to establish the role of MMPs in cerebral ischemic events. Using an animal model, Rosemberg et al[17] demonstrated that MMP-9 activity increases from the first 12 hours after the ischemic event up to the fifth day afterward, whereas MMP-2 increases after the fifth day post-ischemia. A necropsy study performed by Clark et al[18] found maximum MMP-9 activity between the second and fourth days after stroke, with subsequent decreases, whereas MMP-2 activity persisted up to 4 months after the event, with minimum overexpression in the initial phases. In the histologic analysis of plaques classified as unstable, that is, those with ulceration or recent intraplaque hemorrhage, we found a significantly elevated number of cells implicated in cellular immunity (macrophages, T lymphocytes, activated T lymphocytes), as compared with findings in stable plaques. There were no differences in cell numbers in patients examined within the first month after the event and those studied later. Macrophages predominated among the 3 cell types studied, followed by T lymphocytes and activated T lymphocytes. These results support the concept that macrophages are present in all phases of the atherosclerotic process, whereas lymphocytes, activated or not, appear in more advanced phases of the lesions. Studies such as those by Carr et al[4] and Moreno et al[5] support these findings. Carr et al[4] concluded that rupture of the fibrous cap in carotid artery lesions is associated with increased numbers of macrophages and T lymphocytes, which are in an activated state. The activated inflammatory cells could release cytokines or MMPs, which may be responsible for loss of the fibrous cap. Thus inflammation appears to have a role in the pathogenesis of the neurologic symptoms associated with carotid artery stenosis. In a study involving coronary plaques, Moreno et al[5] reported that macrophage-rich areas are more frequently found in patients with unstable angina and non-Q-wave myocardial infarction. This suggests that macrophages are a marker of unstable atherosclerotic plaques and may have a significant role in the pathophysiologic findings in acute coronary syndromes.

The positive correlation we found between the presence of macrophages and MMP-9 overexpression may be attributed to the fact that these cells are potent MMP-9 producers.[19] It seems that the inflammatory phenomena occurring in atherosclerosis could increase MMP expression in the extracellular matrix through cytokine secretion and the presence of macrophages, cells that are infrequently found in normal arterial tissue but that accumulate in areas of the plaque prone to complications.

In the present study, increased MMP-2 levels were found in both symptomatic and asymptomatic disease, but there was no relationship between MMP-2 overexpression and carotid plaque architecture. The finding of MMP-2 overexpression in asymptomatic disease may have to do with the inclusion of patients who had a neurologic event more than 6 months before surgery. MMP-2 increases have been known to persist up to 4 months after the neurologic event.[18] It may be that there was still some residual MMP-2 overexpression in these patients, even though more than 4 months had elapsed.

No correlations were found between elevated MMP-2 levels and unstable plaque or between elevated MMP-2 and the presence of macrophages or T lymphocytes in the plaque. These data lead us to think that the increased levels of this gelatinase in patients with symptomatic disease is probably more related to cerebral injury than to carotid plaque destabilization, in contrast to MMP-9.

It has been proposed that statins can prevent the inflammatory activity that occurs in plaque destabilization. In our series, however, we found no significant differences between patients taking statins and those who did not receive this medication, either in the characteristics of the plaques or in their macrophages or T lymphocyte content.

Blankenberg et al[20] suggested that MMP-9 can be considered a new predictive factor for cardiovascular mortality. In a prospective study including 1127 patients with documented coronary disease, these authors measured basal MMP-9 concentrations and determined MMP-9 genotypes. After a mean follow-up of 4.1 years, they observed that the patients who died of a cardiovascular cause had significantly higher MMP-9 levels than did those who survived. The crude hazard risk ratio of cardiovascular death associated with increasing quartiles of MMP-9 was 1.4 (95% CI, 1.2-1.8; $P < .0001$), and after adjusting for clinical and therapeutic confounders, it was 1.3 (95% CI, 1.1-1.6; $P = .005$). The authors concluded that plasma MMP-9 concentration is a new predictor of cardiovascular mortality in patients with coronary artery disease. Whether it provides independent prognostic information, as compared with other inflammatory markers, will also have to be assessed.

As an added note with regard to the study by Blankenberg et al[20] and other studies, such as those by Loftus et al,[14,15] we mention that the actual reported MMP values in these studies are much lower than those found in the present investigation. We believe the reasons for this discrepancy reside in the different methods used for MMP analysis, and particularly in the different samples analyzed. These other authors measured MMP in plasma, whereas we determined the concentrations in serum. Our rationale for using this sample was to avert variation in the results from incomplete removal or activation of platelets in plasma. Normal MMP values are quite different in plasma and serum; however, our assays recognized both the active and latent total enzyme, which may also have contributed to the difference in the absolute values obtained between the studies. Nevertheless, the concepts coveyed by the results were similar in all 4 works.

In line with the results of these authors, we found elevated MMP-9 in patients with a previous neurologic ischemic event and unstable plaques, and a positive correlation between levels of this gelatinase and the presence of macrophages in the plaque. We believe these findings could have diagnostic and therapeutic implications. Among patients with more than 70% carotid artery stenosis, increased plasma MMP-9 levels could indicate the presence of unstable carotid plaques and identify patients at high risk. Finally, in the same way that platelets became targets for therapeutic interventions when their activity in atherosclerosis was elucidated in the 1970s, the implication of macrophages in the inflammatory mechanisms apparently associated with atherosclerosis may make them potential candidates for a therapeutic approach that will reduce their influence in this disease.

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