Increased plasma levels of metalloproteinase-9 are associated with abdominal aortic aneurysms

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Purpose: **Previous investigators have identified disease-specific elevations of metalloelastase-9 (MMP-9) in aneurysm tissue biopsies. We hypothesized that circulating MMP-9 might also be elevated in patients with aneurysms. The purpose of this study was to compare plasma and aortic tissue MMP-9 levels in patients with infrarenal aneurysms (AAAs), patients with symptomatic aortoiliac occlusive disease (AOD), and healthy patients.**

Methods: **A sandwich enzyme-linked immunosorbent assay was used to measure plasma MMP-9 in patients with AAA (n = 22; mean age, 72.7 years), with AOD (n = 9; mean age, 60.5 years), and without disease (n = 8; mean age, 35.3 years). The MMP-9 levels also were measured in 48-hour supernatants of organ culture tissue explants from patients with AAA (n = 10; mean age, 66.2 years) and AOD (n = 5; mean age, 50.4 years) and organ donors (n = 7; mean age, 48.1 years). The results were reported as the mean ± the standard error of the mean and analyzed with analysis of variance with multivariate regression.**

Results: **The plasma MMP-9 levels were significantly higher in the patients with AAA (85.66 ng/mL ± 11.64) than in the patients with AOD (25.75 ng/mL ± 4.159; $P < .001$) or the healthy patients (13.16 ng/mL ± 1.94; $P < .001$). No significant difference in plasma MMP-9 levels between patients with AOD and healthy patients was identified. The patients with multiple aneurysms had significantly higher levels of plasma MMP-9 than did the patients with an isolated AAA (134.68 ng/mL ± 17.5 vs 71.03 ng/mL ± 10.7; $P < .04$). In organ culture, AAA and AOD tissue explants produced significantly higher levels of MMP-9 (3218.5 ng/gm ± 1115.2 and 1283.1 ng/gm ± 310.6 aortic tissue) than did disease-free explants (6.14 ng/gm ± 2.3 aortic tissue; $P < .0001$). No significant difference in MMP-9 production between AAA and AOD explants was identified.**

Conclusion: **Plasma MMP-9 levels are significantly higher in patients with AAA than in patients with AOD or in healthy volunteers. The patients with multiple aneurysms have higher levels than those patients with an isolated AAA. Organ culture studies suggest that diseased aortic tissue is the source of MMP-9. (J Vasc Surg 1999;29:122-9.)**

Clinicians have long sought a systemic marker for aortic aneurysmal disease. In the past decade, a number of investigators have measured breakdown products of collagen[1] and elastin[2] in the urine and serum of patients with known aortic aneurysms (AAAs) in an effort to identify a reliable marker. Unfortunately, these studies were limited by the fact that only small amounts of such products are released into the circulation and most of the degraded protein remains insoluble within the aortic wall. On the other hand, the enzymes that are responsible for aortic collagen and elastin degradation, the metalloproteinases, are soluble proteins. In 1985, Brown et al[3] recognized elevated levels of an unidentified soluble metalloelastase in the serum of patients with AAA. To date, no other investigator has measured circulating levels of a specific metalloproteinase in patients with aneurysms. Theoretically, a highly expressed soluble metalloproteinase could be continuously released into the systemic circulation and be measurable in the plasma of patients with AAA. If present, the elevations in circulating levels could serve as a marker for the disease in

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patients at risk (ie, those strong family histories) and might correlate with rapid aortic growth.

Although there are extensive studies of metalloproteinases in aortic tissue,[4-10] there is no study that compares circulating levels of the metalloproteinases in patients with and without AAA. Of all the metalloproteinases associated with aortic aneurysms, metalloproteinase-9 (MMP-9) is among the most reasonable enzymes to study. Previous reports from our laboratory and others show that MMP-9 is the principle elastase within the aneurysm wall and that increases in MMP-9 expression exceed those of other metalloproteinases.[4,7,11,12] Therefore, we hypothesize that circulating MMP-9 levels are elevated in patients with AAA and that these elevations also are associated with elevations in MMP-9 production with aneurysmal tissue. The purpose of this study is two-fold:

1. To measure plasma MMP-9 levels in patients before elective aneurysm repair, in patients who are admitted for treatment of symptomatic aortoiliac occlusive disease (AOD), and in healthy volunteers; and
2. To measure aortic tissue MMP-9 production by means of biopsies obtained from patients who undergo aortic surgery for aneurysmal or occlusive disease and from healthy organ donors.

METHODS

Specimens. Two groups of patients were studied. First, plasma was obtained from 22 patients who were admitted for elective repair of AAAs, nine patients who were admitted for treatment of AOD, and eight healthy volunteers after informed consent in accordance with the Institutional Review Board of Northwestern University/McGaw Medical Center. In a second group of patients, aortic tissue samples were obtained at surgery 3 to 6 cm below the renal arteries in 10 patients with infrarenal aortic aneurysms and in five patients with AOD after informed consent in accordance with the Institutional Review Board of Northwestern University/McGaw Medical Center. Seven normal aortic tissue specimens were obtained through the Regional Organ Bank of Illinois from organ donors at the time of tissue procurement. All the patients with a history of other known systemic inflammatory conditions (ie, rheumatoid arthritis, polymyalgia rheumatica, arteritis) or with inflammatory aneurysms were excluded from the study.

Plasma metalloproteinase-9 enzyme-linked immunosorbent assay. Blood samples were collected from venipuncture in heparinized tubes from awake patients before surgery or angiography. Plasma was collected with centrifugation (1000 × *g* for 15 minutes) within 30 minutes of blood sample collection, and the plasma was stored at −80°C until use. A commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit that used a MMP-9 monoclonal antibody (Biotrak MMP-9 Human ELISA System, Amersham Life Science, Buckinghamshire, United Kingdom) was used according to the instructions provided by the manufacturer to determine plasma MMP-9 levels. Specimen MMP-9 levels were quantified via extrapolation from a log-log linear regression curve of purified standards. All the samples were run in duplicate and averaged. If measured MMP-9 levels for an individual sample varied by greater than 10%, the sample was rerun in triplicate and the average value was taken. The plasma samples from the patients with aneurysms were routinely diluted 1:10, and the samples from patients with occlusive disease were diluted 1:2. The samples from the healthy volunteers were not diluted. The samples with MMP-9 levels outside of the linear range of the assay (0.5 to 32 ng/mL) were subsequently serially diluted, and the ELISA was repeated. The within-assay precision (<6%) and the between-assay precision (<10%) were determined by the manufacturer. The specificity of the MMP-9 ELISA as determined by the manufacturer did not reveal cross reactivity with MMP-1, MMP-2, MMP-3, tissue inhibitor of the metalloproteinases–1 (TIMP-1), or TIMP-2. The ELISA measured all forms of MMP-9, including the MMP-9/TIMP-1 complex.

Tissue explant supernatants. The 48-hour tissue supernatants from the operative specimens of AAA (n = 10) and AOD (n = 5) and from the disease-free specimens (n = 7) were collected and processed as previously described.[13,14] Briefly, all the aortic specimens were washed with phosphate-buffered saline solution to remove the residual thrombus, minced, placed into 4 mL/gm of Roswell Park Memorial Institute media (Gibco BRL, Gaithersburg, Mo) with penicillin/streptomycin, and incubated at 37°C with 5% CO_2 for 48 hours. The supernatant then was removed, centrifuged, and stored at −80°C. The MMP-9 levels then were determined with the sandwich ELISA as described above. Aneurysm supernatants routinely were diluted 1:50 to 1:100, occlusive specimen supernatants were diluted 1:20 to 1:50, and healthy aortic supernatants were not diluted.

Data analysis. The results were reported as the mean ± the standard error of the mean and compared with analysis of the variance with a Tukey test to determine the differences between groups. For organ

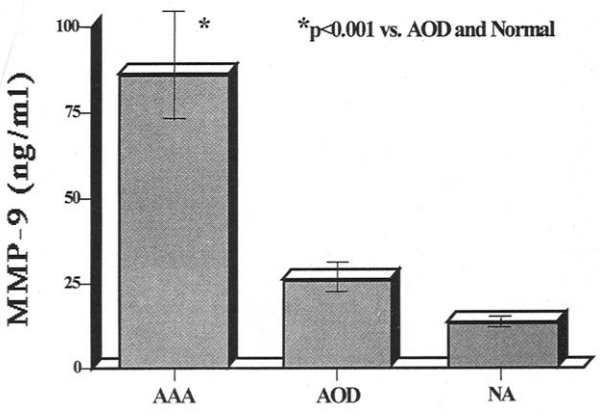

Fig 1. Plasma metalloproteinase levels in patients with abdominal aortic aneurysms and with symptomatic aortoiliac occlusive disease and in healthy volunteers. Metalloproteinase-9 levels were significantly higher in patients with aneurysms ($P < .001$) than in patients with occlusive disease or in healthy volunteers. There was no significant difference between occlusives and normals. Values are expressed as mean ± standard error of mean and compared with analysis of variance with Tukey test.
MMP-9, Metalloproteinase-9; *AAA,* abdominal aortic aneurysm; *AOD,* aortoiliac occlusive disease; *NA,* healthy volunteer.

culture levels, data were logarithmically transformed before the analysis because of non-normal distribution of values. Multivariate regression analysis was used to test for disease characteristics (AAA diameter, presence of other aneurysms) and patient characteristics (family history of AAA, age, sex, smoking) associated with higher MMP-9 plasma levels.

RESULTS

Plasma metalloproteinase-9 levels. Plasma MMP-9 levels were assayed in 22 patients with aneurysms, in nine patients with AOD, and in eight healthy volunteers. The characteristics of each group are listed in Table I. The plasma MMP-9 levels were significantly higher in the patients with aneurysms (85.66 ng/mL ± 11.64; range, 21.2 to 203.8 ng/mL) than in the patients with AOD (25.75 ng/mL ± 4.159; range, 7.9 to 30.5 ng/mL; $P < .001$) or in the healthy volunteers (13.16 ng/mL ± 1.94; range, 7.1 to 21.1; $P < .001$). There was no significant difference identified in the plasma MMP-9 levels between the patients with AOD and the healthy volunteers (Fig 1).

Multivariate regression analysis of all the patients (healthy and with AAA and AOD) with regard to age, sex, smoking, hypertension, and combinations of these factors showed no significant correlation with plasma MMP-9 levels. On the other hand, subgroup analysis of the patients with aneurysms showed significant positive correlation between plasma MMP-9 levels and the presence of more than one aneurysm ($P < .005$), significant negative correlations between the age of the patient with an aneurysm ($P < .04$) and the diameter of the aneurysm present ($P < .05$), and no significant correlation with regard to sex or family history of aneurysm disease. However, when multivariate analysis was applied to all the significant associations in the aneurysm subgroup, only the association between the presence of multiple aneurysms and higher plasma MMP-9 levels remained significant (134.68 ± 17.51 ug/mL vs 71.03 ± 10.73 ug/mL; $P < .04$; Table II).

Tissue explant supernatants. MMP-9 levels were assayed in 48-hour supernatants of tissue explants from 10 aneurysms, five aortas with atherosclerotic occlusive disease, and seven healthy aortas. The patient characteristics of each group are listed in Table I. At 48 hours, the aneurysm tissue explants produced significantly higher levels of MMP-9 (3218.5 ng/gm ± 1115.2 aortic tissue; range, 462 to 10,891 ng/gm) than did the explants from the healthy aorta (6.14 ng/gm ± 2.3 aortic tissue; range, 4.4 to 22.1 ng/gm; $P < .001$). The occlusive tissue explants likewise produced significantly higher levels of MMP-9 (1283.1 ng/gm ± 310.6 aortic tissue; range, 836 to 2480 ng/gm; $P < .001$) than did normal aortic tissue. However, there was no significant difference in MMP-9 production between aneurysm and occlusive aortic tissue explants (Fig 2).

DISCUSSION

Aortic aneurysms are associated with a marked inflammatory infiltrate and evidence of extensive tissue remodeling.[15-18] Although the factors that initiate the formation of the aneurysm are unknown, extensive data exist that detail the complexities and mechanisms responsible for aortic wall remodeling. In particular, aortic aneurysms have been found to have increased levels of several members of the metalloproteinase family (MMP-1, MMP–2, MMP–3, MMP–9).[4-10] These metalloproteinases are important for degradation of the extracellular matrix and weakening of the arterial wall with destruction of both collagen and elastin. Interestingly, the same metalloproteinases are important in matrix remodeling in rheumatoid arthritis and in the spread of metastatic cancer cells.[19,20] In both of these diseases,

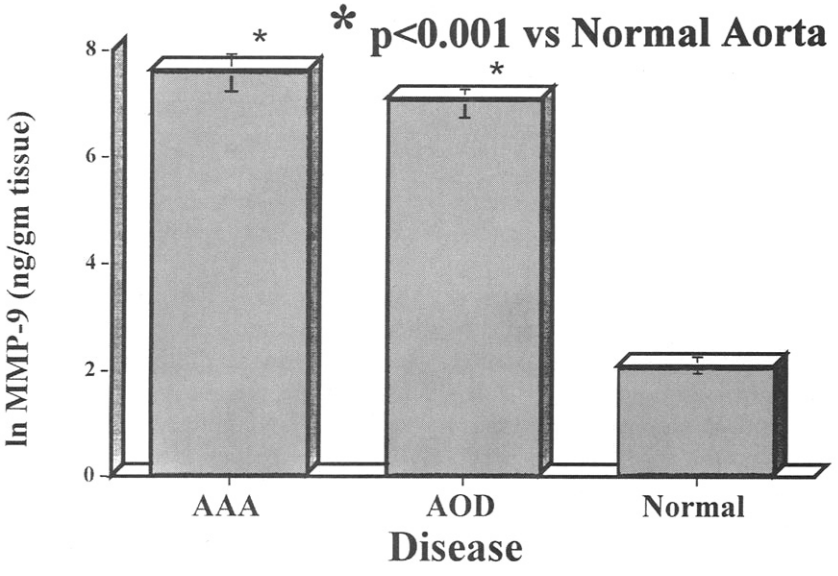

Fig 2. Results of metalloproteinase-9 enzyme-linked immunosorbent assay on 48-hour supernatants from aortic tissue explants. Values were transformed to natural log because of non-normal distribution of data. Aneurysm and occlusive aortic specimens produced significantly more metalloproteinase-9 than did healthy aortic tissue. No significant difference was identified between aneurysm and occlusive specimens.
MMP-9, Metalloproteinase-9; *AAA,* abdominal aortic aneurysm; *AOD,* aortoiliac occlusive disease; *normal,* healthy volunteer.

Table I. Patient characteristics

	AAA plasma (n = 22)	*Occlusive plasma (n = 9)*	*Healthy plasma (n = 8)*	*AAA tissue (n = 10)*	*Occlusive tissue (n = 5)*	*Healthy tissue (n = 7)*
Age (years)	72.7	60.5	35.3	66.2	50.4	48
Sex (M:F)	16:6	7:2	7:1	8:2	4:1	6:2
Hypertension (%)	91	100	0	80%	100%	0%
Smoking (%)	69	71	13	70%	80%	38%
Diabetes (%)	11	44	0	20%	40%	0%

AAA, Abdominal aortic aneurysm.

plasma MMP-9 has been used as a marker for the state of the disease, and in the case of cancer, to detect the presence of occult metastases.[21,22] Because MMP-9 appears to be an important component of the recently described enzyme system responsible for aortic aneurysm formation, it seemed only logical to study circulating plasma levels of this metalloproteinase. In our study, we found that the plasma levels of MMP-9 were significantly elevated in patients with aneurysms, and particularly in those patients with multiple aneurysms, as compared with patients with AOD or with healthy volunteers.

The source of the plasma MMP-9 appears to be the diseased aortic wall. In fact, our organ culture data showed that both aneurysm tissue and occlusive tissue in culture produced between 100 and 1000 times more MMP-9 than did healthy specimens. Our tissue data are in agreement with previous reports from our own laboratory and others that describe elevations in MMP-9 messenger RNA and protein levels in aneurysmal and occlusive tissue biopsies.[8,12] Studies that use in situ hybridization show that MMP-9 is produced by inflammatory cells in both aneurysmal and occlusive tissue sections.[8,10,23,24] Although the aortic tissue in this study was not examined histologically, it may be assumed that inflammation was present in explants from both AAA and AOD. However, the striking

Table II. Regression analysis of 22 patients with aneurysms

Source	*Univariate* P *value*	*Univariate coefficient (continuous variable)*	*Univariate mean (affected vs unaffected)*	*Multivariate* P *value*
Age	0.032	−2.14	NA	.23 (NS)
Other aneurysm	0.006	NA	134.68 vs 71.03	0.033
Diameter	0.049	−18.12	NA	.35 (NS)

difference in the plasma levels of MMP-9 in patients with AAA as compared with AOD stands in apparent contrast to the absence of such a difference in our organ culture data. This difference (plasma values vs tissue culture) probably reflects the greater amount of diseased aortic wall present in patients with AAA as compared with patients with AOD. This notion is further supported by the finding that patients with multiple aneurysms have higher levels than those patients with single aneurysms.

Reports by Powell and others have shown that aneurysms may have varying amounts of particular metalloproteinases on the basis of their size.[9,25] Previous tissue studies show that small aneurysms appear to have greater levels of MMP-2 as compared with MMP-9. In the midrange, 4-cm to 5-cm aneurysms, MMP-9 reaches a peak but later declines with greater enlargement, possibly as a result of burn out of the inflammation within the arterial wall. Although univariate analysis of plasma levels in our study shows a similar trend (ie, a significant negative correlation between plasma MMP-9 level and aneurysm diameter), a wide range of aneurysm size was not available for comparison. Furthermore, it may be that the plasma level does not bear a direct relationship with the size of the aneurysm but rather with the extent of aneurysm. Aneurysms are known for their expansion into the aortoiliac segment, and plasma levels may correlate more directly with the morphology than the absolute diameter of the AAA.

Atherosclerosis and other inflammatory states are associated with nonspecific markers of inflammation.[26] The data presented here regarding MMP-9 may, in part, be a reflection of a similar proinflammatory response. However, in this preliminary study, it seems clear that MMP-9 is associated with aneurysmal disease and that its origin is most likely the arterial wall. Although this study is only a single glimpse of plasma MMP-9 levels in patients who undergo surgical or radiologic intervention, it will be important to study patients who are observed for small aneurysms. Abrupt changes in plasma MMP-9 levels may signal rapid aneurysm expansion, which supports the hypothesis, that the metalloproteinase profile changes with aneurysm size. It has been long assumed that the propensity of aneurysms to rupture is on the basis of diameter, but changes in the biology of the arterial wall (ie, changes in the MMP-9 levels) may play a greater role in the rate of rupture than Laplace's law.

In summary, the results of this study show that MMP-9 levels are significantly elevated in patients with AAAs as compared with patients with symptomatic aortic occlusive disease or with healthy volunteers. Patients with multiple aneurysms have higher circulating levels of MMP-9 than do patients with isolated infrarenal aortic aneurysms. Future longitudinal studies with a large number of patients are necessary to test the clinical value of MMP-9 as an independent marker of aneurysmal disease and to identify patients at risk for rapid expansion of their aneurysms. In addition, it will be important to study circulating levels of MMP-9 in patients after endovascular treatment of their aneurysms and in patients after surgery. Similar to other markers for cancer, such as carotid endarterectomy, the reappearance of this enzyme in the peripheral circulation could signal the development of a recurrent aneurysm.

REFERENCES

1. Satta J, Juvonen T, Haukipuro K, Juvonen M, Kairaluoma MI. Increased turnover of collagen in abdominal aortic aneurysms, demonstrated by measuring the concentration of the aminoterminal propeptide of type III procollagen in peripheral and aortal blood samples. J Vasc Surg 1995; 22:155-60.
2. Cohen JR, Stein TA, Dietzek A, Wise L. Iromaru: -valyl proline in patients with aortic aneurysms. Surg Gynecol Obstet 1989;168:507-12.
3. Brown SL, Backstrom B, Busuttil RW. A new serum proteolytic enzyme in aneurysm pathogenesis. J Vasc Surg 1985;2:393-9.
4. Newman KM, Ogata Y, Malon AM, Irizarry E, Gandhi RH, Nagase H, et al. Identification of matrix metalloproteinases 3 (stromelysin-1) and 9 (gelatinase B) in abdominal aortic aneurysm. Arterioscler Thromb 1994;14:1315-20.
5. Irizarry E, Newman KM, Gandhi RH, Nackman GB, Halpern V, Wishener S, et al. Demonstration of interstitial collagenase in abdominal aortic aneurysm disease. J Surg Res 1993;54:571-4.

6. Newman KM, Malon AM, Shin RD, Scholes JV, Ramey WG, Tilson MD. Matrix metalloproteinases in abdominal aortic aneurysm: characterization, purification, and their possible sources. Connect Tissue Res 1994;30:265-76.
7. Tilson MD, Newman KM. Proteolytic mechanisms in the pathogenesis of aortic aneurysms. In: Yao JST, Pearce WH, editors. Aneurysms: new findings and treatments. Norwalk, Conn: Appleton & Lange; 1994. p. 3-10.
8. McMillan WD, Patterson BK, Keen RR, Shively VP, Cipollone M, Pearce WH. In situ localization and quantification of mRNA for 92kD Type IV collagenase and its inhibitor in aneurysmal, occlusive, and normal aorta. Arterioscler Thromb Vasc Biol 1995;15:1139-44.
9. Freestone T, Turner RJ, Coady A, Higman DJ, Greenhalgh RM, Powell JT. Inflammation and matrix metalloproteinases in the enlarging abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol 1995;15:1145-51.
10. McMillan WD, Patterson BK, Keen RR, Pearce WH. In situ localization and quantification of seventy-two–kilodalton type IV collagenase in aneurysmal, occlusive, and normal aorta. J Vasc Surg 1995;22:295-305.
11. Tamarina NA, McMillan WD, Shively VP, Pearce WH. Expression of metalloproteinases and their inhibitors in aneurysms and normal aorta. Surgery. In press.
12. Sakalihasan N, Delvenne P, Nusgens BV, Limet R, Lapière CM. Activated forms of MMP_2 and MMP_9 in abdominal aortic aneurysms. J Vasc Surg 1996;24:127-33.
13. Shireman PK, McCarthy WJ, Pearce WH, Shively VP, Cipollone M, Kwaan HC. Elevations of tissue-type plasminogen activator and differential expression of urokinase-type plasminogen activator in diseased aorta. J Vasc Surg 1997;25:157-64.
14. Shireman PK, McCarthy WJ, Pearce WH, Patterson BK, Shively VP, Cipollone M, et al. Elevated levels of plasminogen-activator inhibitor type 1 in atherosclerotic aorta. J Vasc Surg 1996;23:810-8.
15. Glagov S. Morphology of collagen and elastin fibers in atherosclerotic lesions. Adv Exp Med Biol 1975;82:767-73.
16. Sumner DS, Hokansen DE, Strandness DE. Stress-strain characteristics and collagen-elastin content of abdominal aortic aneurysms. Surg Gynecol Obstet 1970;130:459-66.
17. Campa JS, Greenhalgh RM, Powell JT. Elastin degradation in abdominal aortic aneurysms. Atherosclerosis 1987;65:13-21.
18. Vine N and Powell JT. Metalloproteinases in degenerative aortic disease. Clin Sci 1991;81:233-9.
19. Unemori EN, Hibbs MS, Amento EP. Constitutive expression of a 92kD gelatinase (type V collagenase) by rheumatoid synovial fibroblasts and its induction in normal human fibroblasts by inflammatory cytokines. J Clin Invest 1991; 88:1656-62.
20. Moscatelli D, Rifkin DB. Membrane and matrix location of proteinases, a common theme in tumor cell invasion and angiogenesis. Biochim Biophys Acta 1988;948:67-85.
21. Vartio T, Baumann M. Human gelatinase/type IV procollagenase is a regular plasma component. FEBS Lett 1989;255: 285-9.
22. Zucker S, Lysik RM, Zarrabi MH, Moll U. Mr 92,000 type IV collagenase is increased in plasma of patients with colon cancer and breast cancer. Cancer Res 1993;53:140-6.
23. Newman KM, Jean-Claude J, Li H, Scholes JV, Ogata Y, Nagase H, et al. Cellular localization of matrix metalloproteinases in the abdominal aortic aneurysm wall. J Vasc Surg 1994;20:814-20.
24. Herron GS, Unemori E, Wong M, Rapp JH, Hipps MH, Stoney RJ. Connective tissue proteinases and inhibitors in abdominal aortic aneurysms. Arterioscler Thromb 119;11: 1667-77.
25. McMillan WD, Tamarina NT, Cipollone M, Johnson DA, Parker MA, Pearce WH. Size matters: the relationship between MMP-9 expression and aortic diameter. Circulation 1997;96:2228-32.
26. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336: 973-9.

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DISCUSSION

Dr Robert W. Thompson (St Louis, Mo). Abdominal aortic aneurysms are associated with chronic inflammation and connective tissue destruction. It is now almost established dogma that matrix metalloproteinases participate in the pathogenesis of aneurysm disease. MMP-9 is one of 18 different matrix metalloproteinases, but it is of particular interest in aneurysms for the following reasons: (1) it is an inducible product of macrophages that are prevalent within aneurysm tissues; (2) it is one of only four MMPs capable of degrading elastin fibers, at least in vitro; and (3) it is produced in large amounts in human and experimental abdominal aortic aneurysms. Drs McMillan and Pearce have now begun to address another dimension of this problem by examining whether the production of MMP-9 in aneurysms is high enough to result in elevated circulating levels. I think this paper will be remembered because it shows for the first time that plasma MMP-9 is convincingly elevated in patients with aneurysms as compared with appropriate controls. This initially does not appear to be particularly surprising; however, it has important implications for further investigation.

First, the authors suggest that elevated plasma levels of MMP-9 are derived from the aneurysm itself. They base this conclusion on studies in organ culture. Their results are remarkably similar to those we reported 3 years ago in the Journal of Clinical Investigation, with a similar enzyme-linked immunosorbent assay for MMP-9. On the other hand, MMP-9 is normally present in plasma and it is produced in many other tissues, including atherosclerotic plaques. And it is increased in the plasma in diseases associated with chronic inflammation, particularly rheumatoid arthritis and cancers of the breast and gastrointestinal tract. Speculation as to the source of circulating MMP-9 must therefore be interpreted with some degree of cau-

tion. If the aneurysm was indeed the source of circulating MMP-9 in these patients, one would predict that plasma MMP-9 levels would fall after aneurysm repair. Because this would provide more convincing evidence, have the authors taken the opportunity to remeasure plasma MMP-9 after surgical repair? And, if so, did they observe the expected decrease that they might have found?

My second question arises from the suggestion that plasma MMP-9 might be used as a screening test to determine which patients might be prone to the development of aortic aneurysms. I wonder whether there is really a need for such a test. We are already aware of many clinical factors that raise susceptibility to aneurysm disease, particularly age more than 65 years, atherosclerosis, and smoking. Six percent to 9% of the population with these risk factors will be found to have an aortic aneurysm with ultrasound scan screening. Abdominal ultrasound scanning is clearly more accurate in determining whether an aneurysm is present, and my suspicion is that a plasma enzyme-linked immunosorbent assay for MMP-9 is not going to be all that different in cost than an ultrasound scan when used as a population screening test. It is also unclear how one might deal with the fact that MMP-9 levels are elevated in a number of other diseases. Can the authors speculate on the potential cost of a plasma MMP-9 enzyme-linked immunosorbent assay measurement and discuss how one would evaluate a patient's levels with a positive blood test but no abdominal aneurysm with ultrasound scan?

Additional concerns in using plasma MMP-9 for screening are that the number of patients in this study was relatively small compared with other studies on plasma MMP-9 in other conditions and that the mean age of the control group was less than the age in the aneurysm group. Also, this study does not provide evidence that MMP-9 is elevated either before the development or early in the development of aortic aneurysms nor does it show that MMP-9 actually plays a direct role in causing aneurysmal degeneration. Like other proteins, plasma MMP-9 levels are simply elevated in the patients with established aneurysms. Circulating levels of tumor necrosing factor–α, interleukin-1, γ-interferon, and, most particularly, the aminoterminal propeptide of type III procollagen also are markedly elevated in these patients. These are all markers of chronic inflammation and accelerated connective tissue metabolism. I wonder whether the authors have had a chance to examine how the plasma levels of MMP-9 compare with these other types of circulating markers of inflammation.

Finally, I think the real reason this study is so important can actually be drawn from the paper by Dr Bigatel and colleagues. For example, as we begin to decipher the mechanisms responsible for aneurysm growth and as this knowledge becomes translated into new pharmacologic strategies for small aortic aneurysms, there will be an increasing need for simple assays to monitor the patient's response to therapy. If MMP-9 is derived from the aneurysm wall and if it is elevated in patients with small aortic aneurysms, assays like this might provide a valuable way to monitor disease activity in patients who are treated with drugs like doxycycline and other metalloproteinase inhibitors rather than depend solely on ultrasound scan examination at 6-month to 12-month intervals. In this light, my final question is whether the authors have measured plasma MMP-9 levels in patients with small aortic aneurysms and have they observed any changes in the patients who have been followed without surgical repair, especially during gradual aneurysm expansion?

I think we all look forward to further studies on this problem, and I thank the Society for the opportunity to discuss this intriguing paper.

Dr William D. McMillan (Chicago, Ill). Thank you, Dr Thompson, for those insightful comments.

I did have one slide prepared because I anticipated one question about whether or not the levels fell after the patients underwent repair. This really represents preliminary data that I have only collected on five of the 22 patients in the initial study. But one gets the idea that, in each of these five patients, each of the levels fell, and, in fact, each returned to the normal range. And so, I feel somewhat cautiously optimistic that the levels will return to normal after repair, which indicates in some way, albeit it somewhat superficially, that the aneurysm may be the source of the enzyme in the bloodstream.

The second question asked was whether or not this was really going to work out as a screening test. I would try to say that our point in doing this work was not to advocate it as a screening test for aneurysms. There are a variety of other issues involved in that.

How much does it cost? I can tell you that the enzyme-linked immunosorbent assay kit costs $500 and that you can examine about six patients on a single kit. Certainly, the cost would be lessened if the examination were done in large scale, but I do not really have a handle on whether or not it would be cost effective as compared with an ultrasound scan.

In terms of the longitudinal evaluation of patients, it is intriguing to wonder whether or not, if you followed a patient with a small aneurysm, an elevated MMP-9 level would signal a more rapid expansion rate. And certainly that would be one of our long-term goals in measuring this enzyme. I think that, as Dr Thompson correctly pointed out, the screening of large populations with this blood test may not be where this is headed at all. On the other hand, it may be possible to find more clinically relevant correlations with expansion rate or degree of inflammation with the MMP-9 levels.

Finally, with regards to the other studies that are out there in terms of markers for aneurysm disease, I like our study a little better, not because I doubt the data of the other authors, but because mechanically this makes more sense to me. The MMP-9 is a potential degradative enzyme that can cause aneurysms at least in experimental models. And the fact that it goes away with the repair of the aneurysm is again suggestive that it actually does come from the aneurysm.

We do not have data about levels in patients with small aneurysms who have been followed longitudinally.

Dr B. Timothy Baxter (Omaha, Neb). Congratulations on your study. My question is, Have you considered the possibility that the high MMP-9 levels were related to thrombus in the aneurysm? Did you correlate this with thrombus to see if that was a possibility?

Dr McMillan. I did do that about a month and a half ago. I went back and looked at as many computed tomographic scans as I could find of these patients, and I did not find a correlation that was significant between the amount of thrombus. Now, again, we are dealing with a relatively small number of patients and I did not have all the computed tomographic scans available to make that determination.

Dr Glenn C. Hunter (Galveston, Tex). I enjoyed your paper. I have a couple of questions. One relates to your control group. I assume these specimens were obtained from transplant donors who receive numerous medications and preservation solutions that may potentially influence your assay. I wonder, therefore, how certain you are that the controls do not express MMPs in organ culture?

Secondly, have you examined these same specimens histologically to assess the degree of inflammation present? If you postulate that the aorta is producing the MMPs, then there should be a large number of inflammatory cells in the tissue.

Dr McMillan. As to the first question about the control group and the tissue studies, we go to the actual organ harvest to obtain these and get them out of the patient as soon as possible, during the time that the kidneys and liver and pancreas are harvested. So, as best as we can, we try to avoid long delays from the procurement to our actual analysis of the tissue.

As to the second question, we and others have looked in previous studies at the amount of inflammation within the aortic wall and have found that the aneurysms have higher inflammatory scores and higher levels of MMP-9 in their wall. We did not repeat that in this study, and, therefore, I have to answer that from previously published data.

Dr William H. Baker (Maywood, Ill). This was a very nice paper. The Northwestern group has had a unique opportunity to follow patients who have undergone endovascular repair. Have you done so with this technique?

Dr McMillan. Yes, we are trying to do that. I have, like a vampire, collected five specimens from patients who have undergone endovascular repair and have gotten two of them back in follow-up examination. Both of the levels fell, but I cannot correlate beyond that. And certainly that is something that will be interesting to see, whether the patient with a continually expanding aneurysm or one that does not shrink has an elevated level as compared with a patient who has a completely excluded and shrinking aneurysm.

Dr Wiley F. Barker (Los Angeles, Calif). To clarify that last point, your last slide indicated this fall in levels of MMP-9 after treatment of the aneurysms. Were those aneurysms treated with complete excision or not?

Dr McMillan. There were five patients. Three of them underwent treatment with actual open repair with a graft placed, and two of them underwent treatment by endovascular means.

Dr Christopher K. Zarins (Stanford, Calif). The fact that you found elevated MMP-9 levels in both aneurysmal and occlusive tissue is interesting. Does that allow you to address the age-old question on the pathogenesis of aneurysms? Is it related to atherosclerosis?

Dr McMillan. I believe that it does not give me a license to address that question conclusively. My personal feeling is that it is related to atherosclerosis, but beyond that I cannot give you a strong answer.