Plasma Levels of Metalloproteinases-3 and -9 as Markers of Successful Abdominal Aortic Aneurysm Exclusion After Endovascular Graft Treatment

Giuseppe Sangiorgi, MD, FESC; Rita D’Averio, MD, PhD; Alessandro Mauriello, MD; Monya Bondio, MD; Marina Pontillo, PhD; Serenella Castelvecchio, MD; Santi Trimarchi, MD; Valerio Tolva, MD; Giovanni Nano, MD; Vincenzo Rampoldi, MD; Luigi Giusto Spagnoli, MD; Luigi Inglese, MD

Background—Structural alterations of aortic wall resulting from degradation of matrix proteins by matrix metalloproteinases (MMPs) characterize abdominal aortic aneurysms (AAAs). No studies have compared circulating levels of MMPs after endovascular graft (EVG) exclusion in comparison with open surgical repair (OSR) in patients affected by AAA.

Methods and Results—An abdominal angiography and CT scan were performed in all patients at the time of enrollment. A spiral CT scan was performed at 6 months to detect presence of endoleaks. MMP-3 and MMP-9 levels were measured before EVG (n=30) and OSR (n=15) treatments and at 1, 3, and 6 months of follow-up by a sandwich ELISA technique. Healthy volunteers (n=10) were used as control subjects. Immunohistochemical staining for MMP-9 and MMP-3 was performed on tissue samples from surgical cases. Both MMP-9 and MMP-3 mean basal levels were significantly higher in patients affected by AAA than in control subjects (32.3±20.7 ng/mL for EVG and 28±9.9 ng/mL for OSR versus 8.9±2.5 ng/mL, 2P<0.05; 18.3±9.7 ng/mL and 26.7±10.8 ng/mL versus 8.2±5.3 ng/mL, 2P<0.001). In the OSR group, both MMP-9 and MMP-3 mean levels decreased after surgery (28±9.9 ng/mL at basal versus 14.7±6.6 ng/mL at 6 months, 2P<0.001; 26.7±10.8 versus 12±5.3 ng/mL; 2P<0.001). In the EVG group, a statistically significant difference at 6-month follow-up in MMP-9 and MMP-3 mean plasma values was detected in patients who had endoleakage in comparison with patients without endoleakage (44.3±20.7 versus 14.6±7.0 ng/mL, 2P<0.005; 25±11.5 versus 10.3±5.4 ng/mL, 2P<0.005).

Conclusions—After EVG exclusion, MMP-9 and MMP-3 levels decreased to a level similar to that of patients undergoing OSR. In addition, a lack of decrease in MMP levels after EVG exclusion may help in identifying patients who will have endoleakage and consequent aneurysm expansion caused by continuous sac pressurization during follow-up. (Circulation. 2001;104[suppl I]:I-288-I-295.)

Key Words: aneurysm ■ metalloproteinases ■ peripheral vascular disease

A bdominal aortic aneurysm (AAA) is a common degenerative condition with an estimated incidence of 20 to 40 cases per 100 000, and nearly 45 000 surgical operation are performed annually in the United States for the treatment of this potentially lethal disease.1,2 Different histomorphological studies have demonstrated that aneurysmal tissue is characterized by elevation in elastolytic and collagenolytic activity caused by increase in production of matrix metalloproteinase (MMP) enzymes when compared with normal aortic tissue.3-7 The progressive degradation of fibrillar matrix proteins of the elastic media and outer aortic wall has been identified as the putative process that results in AAA formation.3,8-11 In particular, through the use of immunohistochemical and in situ zimography techniques, several elastolytic matrix MMPs have been related to AAA expansion and eventual rupture, including gelatinase A (72-kDa type IV collagenase; MMP-2), gelatinase B (92-kDa type IV collagenase, MMP-9), and stromelysin (MMP-3).12-14 Unfortunately, these techniques rely on aortic tissue analysis and therefore could only be performed on surgical samples. Recently, McMillan and Pearce15 have demonstrated that highly expressed soluble MMP-9 can be measurable in the plasma of patients affected by AAA by a simple ELISA technique.

Currently, no medical therapy can prevent aneurysm growth and decrease the risk of rupture. Open surgical repair (OSR) of the diseased aortic segment with a prosthetic graft represents the standard of therapy for AAA. Pooled data from
leading medical centers and population-based studies document mortality rates of 4% to 6% for elective aortic reconstruction, whereas operative mortality rate for ruptured aneurysm remains ~50%. Against this background of contemporary surgical management of aortic aneurysm, exclusion of AAA by endovascular graft (EVG) placement has been recently demonstrated to be a valid therapeutic strategy in alternative to surgical repair.19–21 Although stent grafts for AAA repair are feasible and reasonably safe in the short-term, with less than 1% conversion rate to open repair and a 30-day mortality rate of 1% in most recent series,22,23 the intermediate and long-term efficacy remains uncertain. In particular, an aneurysm rupture risk of 0.7% from 1 to 24 months after successful endovascular repair has been reported by Zarins and associates.24 In addition, several authors have shown that after endovascular exclusion, there are still 30% to 40% of cases in which a persistent endoleakage, associated with continuous sac pressurization, aneurysm growth, and increased risk of rupture, is present at 1-year follow-up.21,25–28

To date, clinical diagnosis of endoleakage relies exclusively on multiple CT scan examinations during follow-up and additional angiographic evaluation for leakages thought to be relevant,29–31 with additional clinical management cost expenditure. No study has compared circulating levels of MMPs after EVG exclusion of AAA. If present, the elevations of MMPs in the plasma could serve as a marker of persistent endoleakage. Conversely, decrease in the amount of circulating MMPs could represent a simple marker of successful aneurysm exclusion. Therefore, the objectives of this study were (1) to assess circulating levels of MMP-3 and MMP-9 in patients affected by AAA and submitted to either endovascular or surgical repair compared with healthy volunteers, and (2) to evaluate if plasma levels of MMPs can represent a reliable marker of endoleakage presence after EVG placement up to 6 months after deployment.

**Methods**

**Selection of Patients**

Between August 1999 and October 2000, 75 patients affected by AAA were admitted to our hospital to detect the extension and morphology of the diseased aortic segment. All patients underwent standard preoperative evaluation unless emergency situation (eg, symptomatic or leaking aneurysm) demanded urgent treatment that could not be delayed by certain preoperative tests. Risk assessment criteria were based on preoperative medical history, full physical examination, biochemical and hematological analyses, echocardiography, ECG, and lung function tests. Nine patients were symptomatic for abdominal pain at the time of hospital admission. The clinical characteristics of patients treated by EVG placement or OSR are summarized in Table 1. No differences were detected between groups and control subjects regarding age, sex, cardiovascular risk factors, American Society of Anesthesiologists score, or other comorbidity.

All patients underwent basal abdominopelvic CT scan with evaluation of aneurysm length, diameter, quality, and dimension of the proximal and distal necks, diameter of the access vessels, and presence or absence of mural thrombus at the necks. If it appeared that the patient could be a candidate of endovascular grafting on the basis of the CT finding, a contrast abdominal aortogram with a calibrated catheter, which contained markers at 1-cm intervals, was performed, and the mesenteric circulation as a whole, lumbar arteries, and any other aberrant vessels were evaluated. AAs were classified according to the anatomic classification proposed by Schumacher and associates.33 Patients with aneurysm diameter >4.0 cm or documented aneurysm expansion >5 mm in 12 months were considered eligible for EVG treatment.19,34

Dimensional exclusion criteria for EVG candidates included a proximal aortic neck diameter <20 mm, or <1.5 cm of nonaneurysmal infrarenal aorta at the proximal attachment site. Patients with angulation of the aorta >60°, aberrant renal arteries, or tortuosity and kinking of the iliac vessels were also excluded from endovascular repair and referred for conventional OSR.

### Table 1. Population Clinical Characteristics of Patients Undergoing Either Surgical or Endovascular Abdominal Aortic Aneurysm Repair and of Control Subjects

<table>
<thead>
<tr>
<th>Clinical Variables</th>
<th>Surgical Group (n=15)</th>
<th>EVG Group (n=30)</th>
<th>Control Subjects (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65±8.4</td>
<td>66±5.2</td>
<td>63±10</td>
<td>NS</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>12 (80)</td>
<td>24 (80)</td>
<td>6 (60)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>3 (20)</td>
<td>3 (10)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>2 (13.3)</td>
<td>4 (13.3)</td>
<td>1 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>9 (60)</td>
<td>13 (55)</td>
<td>4 (40)</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>3 (20)</td>
<td>4 (43.3)</td>
<td>2 (20)</td>
<td>NS</td>
</tr>
<tr>
<td>Ischemic heart disease, n (%)</td>
<td>5 (50)</td>
<td>4 (13.3)</td>
<td>1 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Previous CABG, n (%)</td>
<td>3 (20)</td>
<td>7 (23.3)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Previous vascular surgery, n (%)</td>
<td>2 (13.3)</td>
<td>6 (30)</td>
<td>1 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Renal insufficiency (creatinine &gt;150 μmol/L), n (%)</td>
<td>1 (6.6)</td>
<td>4 (13.3)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease, n (%)</td>
<td>6 (40)</td>
<td>12 (40)</td>
<td>1 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>ASA score</td>
<td>1.1±0.9</td>
<td>1.2±1.1</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>Symptomatic aneurysm, n (%)</td>
<td>3 (20)</td>
<td>4 (13.3)</td>
<td>...</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are reported as mean±SD or n (%). ASA indicates American Society of Anesthesiologists.
Thirty patients were excluded from the study for a positive history of lipid-lowering drug administration, which can potentially affect MMP determination. Of the remaining, 30 patients fulfilled endovascular treatment criteria and were treated by endovascular stent placement (EVG group). Fifteen patients were treated with OSR (OSR group). In addition, 10 healthy, age-matched volunteers were used as control subjects.

Approval for the study had been obtained from the institutional review board, and informed consent was granted by each patient.

Stent Graft Placement

All graft implantations were performed in an operating room by a team of vascular surgeons and interventional cardiologists. All procedures were performed under spinal anesthesia.

Details regarding the endovascular procedure, the deployment of the stent graft, and postprocedural management have been reported previously in details. Patients were given aspirin after endovascular treatment. The median operation time was 150 minutes (106 to 210 minutes), and the fluoroscopy time was 32 minutes (18 to 42 minutes).

MMP Plasma Measurements

Blood samples were obtained from venipuncture in EDTA tubes from fasting patients either before EVG placement or OSR and at 1-, 3-, and 6-month follow-up intervals. Plasma was collected with centrifugation (3000 g for 20 minutes), and the plasma was stored at −80°C until use. MMP levels were determined by commercially available sandwich ELISA kits, which have been validated for use with human tissue homogenates, with an MMP-9 monoclonal antibody (Biotrak MMP-9 Human ELISA system, Amersham Life Science) and an MMP-3 monoclonal antibody (Bindazyme pro-MMP-3 Human ELISA system, The Binding Site Ltd), according to the instructions provided by manufacturers. The ELISA kit measured both pro-MMP-9, pro-MMP-3, and MMP-9/pro-MMP-3 tissue inhibitor of the metalloproteinase 1 (TIMP-1) or TIMP-2 complex at 10% for both MMP-9 and MMP-3, and the between-assay precision (7% for MMP-9, 13.2 months.

MMP-9 Level Determination

The assay was based on 2-site ELISA sandwich format with 2 antibodies directed against different epitopes of MMP-9. During the first incubation step, the MMP-9 was bound to the antibody-protein A microplate. Then, during the second incubation step, detection antibody conjugated with horseradish peroxidase was added, with formation of an immobilized complex. The amount of peroxidase bound to each well was determined by the addition of 3.3',5',5' tetramethylbenzidine (TMB) substrate. The kit is able to detect plasmatic concentration between the range of 4 to 128 ng/mL, as described by the manufacturer. All reagents were equilibrated to a temperature of 20° to 27°C and the plate read at 450 nm with a spectrophotometric plate reader. The sensitivity, defined as 2 SD above the zero dose binding, was determined as 0.6 ng/mL. The data were determined by the manufacturer with the use of the assay designed for MMP-9 in tissue and cell cultures.

MMP-3 Level Determination

Microwells were precoated with affinity-purified antibody to pro-MMP-3; then diluted test specimens were added to the wells allowing the pro-MMP-3 to bind to the coating antibody during the first incubation. The wells were then washed to remove unbound proteins, and a biotinylated affinity-purified antibody to pro-MMP-3 was added. The latter, during subsequent incubation, bound to the captured pro-MMP-3. Excess antibody was removed by a further wash step, and streptavidin peroxidase was added. After further incubation, the excess enzyme conjugate was removed by washing, and the bound conjugate was visualized with TMB substrate. The plate was read at 450 nm with a spectrophotometric plate reader. The analytic sensitivity was determined as the mean concentration ±2 SD given by 20 determinations of the calibrator diluent. This equates to 2.1 ng/mL with the use of 1:20 sample dilution.

Immunohistochemical Study

Aneurysmal aortic specimens were obtained at the time of surgical operation. All aneurysm tissue was obtained 3 to 6 cm below the renal arteries. Tissue was fixed in 10% formalin, embedded in paraffin, and stained with the Movat pentachrome method. The immunohistochemical study was used to characterize the expression of metalloproteinases in aneurysmal aortic specimens. Serial sections 5 mm thick were incubated with MMP-3 and MMP-9 antibodies (Calbiochem) and detected by the streptavidin/biotin immunoperoxidase method with diaminobenzidine used as the chromogen substrate. Additional controls were performed by omitting primary or secondary antibody. Immunohistochemical data were described qualitatively in a blinded fashion by 2 pathologists; no staining was recorded as 0; weak, patchy staining as 1; moderate, patchy staining as 2; moderate, diffuse staining as 3; and strong, diffuse staining as 4.

Follow-Up Imaging and Analysis

The follow-up protocol included a physical examination, laboratory evaluation, and an abdominal CT scan before discharge from the hospital. Spiral CT scans were then obtained 6 months after treatment and at the onset of any new symptoms. At the CT scan examination, the prime imaging parameters determined were (1) the maximum transverse diameter of the aneurysm and (2) the presence or absence of perigraft endoleakage within the aneurysmal sac. Endoleakage was defined by persistence of blood flow outside the lumen of the endoluminal graft but within the aneurysm sac or adjacent vascular segment being treated by the graft. Endoleaks were divided in early, existing immediately after the initial procedure, and late, which develop de novo during follow-up. If an endoleak was discovered after EVG placement, it was followed up closely and treated if the size of the aneurysm enlarged in comparison with preprocedural size. Accordingly, MMP plasma levels were measured at 6 months after endoleak treatment.

Early mortality and morbidity included events occurring within 30 days after the EVG or OSR procedure, either in the hospital or after hospital discharge. Information about the patient was compiled from chart review and by contacting the patients or their treating physician. Final follow-up was completed in all patients; the mean duration of follow-up was 6.3 months and the maximal duration was 13.2 months.

Statistical Analysis

Data were analyzed by SPSS (Statistical Package for the Social Sciences) software. All data are presented as mean±SD. The Pearson x² test was used to assess possible differences for clinical variables between the EVG, surgical, and control groups. A 2-tailed unpaired Student’s t test was used to compare angiographic and CT scan measurements between EVG and surgical groups and to assess differences in MMP levels. A 2-tailed paired Student’s t test was used to compare MMP levels within the same group at different time points. Univariate and multivariate linear regression analyses and Pearson correlation coefficient (r) were used to identify any significant associations between MMP-9 and MMP-3 plasma levels and the clinical characteristics of patient populations. Statistical significance was set at 2P<0.05.

Results

CT Scan and Abdominal Angiography Data

Preprocedural and postprocedural CT scan measurements and abdominal angiographic variables are summarized in Table 2. No statistically significant differences were detected in the 2 study groups regarding the different variables measured by angiography or CT scan imaging. Mean maximum transverse
Table 2. Angiographic and CT Scan Measurements in Study Population of Patients Undergoing Surgical or Endovascular Abdominal Aortic Aneurysm Repair

<table>
<thead>
<tr>
<th>Measured Variables</th>
<th>Surgical Group (n=15)</th>
<th>EVG Group (n=30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal neck length, mm</td>
<td>29.1±13.4</td>
<td>31.8±22.6</td>
<td>NS</td>
</tr>
<tr>
<td>Distal neck length, mm</td>
<td>25.9±20.3</td>
<td>26.3±17.8</td>
<td>NS</td>
</tr>
<tr>
<td>Proximal neck diameter, mm</td>
<td>24.2±11.8</td>
<td>23.4±6.5</td>
<td>NS</td>
</tr>
<tr>
<td>Distal neck diameter, mm</td>
<td>16.8±2.3</td>
<td>20.1±10.4</td>
<td>NS</td>
</tr>
<tr>
<td>Basal aneurysm MTD, mm</td>
<td>52.3±9.7</td>
<td>45.8±10</td>
<td>NS</td>
</tr>
<tr>
<td>Follow-up aneurysm MTD, mm</td>
<td>. . .</td>
<td>42.2±11.5</td>
<td>. . .</td>
</tr>
<tr>
<td>Endoleak</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early, n (%)</td>
<td>. . .</td>
<td>1 (3.3)</td>
<td>. . .</td>
</tr>
<tr>
<td>Late, n (%)</td>
<td>. . .</td>
<td>6 (20)</td>
<td>. . .</td>
</tr>
</tbody>
</table>

Data are reported as mean±SD or n (%). MTD indicates maximum transverse diameter.

The diameter of AAA was 52.3±9.7 mm in the OSR group and 45.8±10 mm in the EVG group. The CT scan maximum transverse diameter of AAA measurement in the EVG group decreased from 45.8±10 mm to 42.2±11.5 mm at 6-month follow-up (P<0.006).

EVG Data

Three types of commercially manufactured bifurcated devices for stent graft treatment were used: the Gore Excluder graft (W.L. Gore & Associates; n=16, 53.4%), the Vanguard graft (Boston Scientific Corp; n=7, 23.3%), and the Talent graft (World Medical; n=7, 23.3%). All endografts were deployed successfully as planned with no conversions to OSR. Five patients (16.6%) required 8 additional endovascular stents (Palmaz or Wallstent) to correct stenoses of the iliac arteries and renal arteries. Adjunctive coil embolization was performed in 8 patients (26.6%) with large patent lumbar or large patent inferior mesenteric arteries.

Primary technical success, defined as angiographic complete aneurysm exclusion with continuous aortic patency, was achieved in all patients (100%). One patient (3.3%) in the EVG group had pneumonia and 1 patient had kidney failure (3.3%) requiring hemodialysis for 2 weeks. Postoperative discharge averaged 3 days. There were no major complications as defined by myocardial infarction, stroke, renal dysfunction, or lower-extremity ischemia during follow-up. Six-month mortality rate was zero in both groups.

Periprosthetic leakages were identified in 7 patients (23.3%) during follow-up. The site of the leak was at the proximal attachment site in 2 patients (6.6%, Figure 1), and one resolved spontaneously within 1-month follow-up. The other patient was treated 28 weeks after with placement of an aortic cuff (Talent, Medtronic Inc) proximal to the endoleakage site. Two patients (6.6%) showed periprosthetic flow arising in the mid portion of the aneurysm sac caused by retrograde flow from a lumbar artery and are being followed up closely to monitor the status of the aneurysm and the leakage. Three patients (10%) had late leakages at the distal attachment site that were treated 20 weeks after aneurysm repair by placement of a Wallstent (Boston Scientific Corp) across the distal attachment.

MMP Quantification by ELISA Technique

Mean preoperative plasma value for MMP-9 was 3 times higher in patients affected by AAA than in control patients (32.3±20.7 ng/mL for the EVG group and 28±9.9 ng/mL for the OSR group versus 8.9±2.5 ng/mL, 2P<0.05; respectively). Similarly, MMP-3 mean basal value was significantly higher in EVG and surgically treated groups compared with healthy volunteers used as control subjects (18.3±9.7 ng/mL and 26.7±10.8 ng/mL versus 8.2±5.3, respectively, 2P<0.001). In the surgical group (n=15), both MMP-9 and MMP-3 mean levels at 6 months decreased significantly in comparison with preoperative levels (14.7±6.6 ng/mL versus 28±9.9 ng/mL; 12±5.3 ng/mL versus 26.7±10.8 ng/mL; 2P<0.001, respectively, Table 3). In the EVG group (n=30), MMP-9 mean plasma values increased during follow-up in patients who had endoleakage after EVG placement (n=7, 23.3%) in comparison with patients (n=23, 76.6%) without endoleakage (35.4±24.2 ng/mL versus 24.5±15.2 ng/mL at 1 month, 1P=NS; 40.2±20.9 ng/mL versus 23.6±10.4 ng/mL at 3 months, 2P<0.005; 44.3±20.7 ng/mL versus 14.6±7.0 ng/mL at 6 months, 2P<0.005, respectively, Figure 2 and Table 3). Similarly, MMP-3 mean plasma levels were significantly decreased at 6-month follow-up in patients with no endoleaks compared with patients showing endoleakage (10.3±5.4 ng/mL versus 25±11.5 ng/mL, 2P<0.005; Figure 2). In addition, 6 months after endoleakage treatment in 5 patients, there was a significant decrease in MMP-9 and MMP-3 plasma levels compared with the value before treatment (9.9±1.6 ng/mL versus 44.3±20.7 ng/mL for MMP9; 10.8±2.3 ng/mL versus 25±11.5 ng/mL for MMP-3, 1P<0.0001; respectively), suggesting complete exclusion of the aneurysm at this time.
**MMPs, AAAs, and Surgical Treatment**

Metalloproteinases are a family of related zinc metalloendopeptidases that play an important role in the turnover of components of the extracellular matrix.8 In particular, several histological studies have demonstrated that AAA tissue, compared with normal aortic tissue, contains an excess of several members of MMPs, including MMP-1, MMP-2, MMP-3, and MMP-9.4,11,13,14

A single study, performed with the same ELISA technique used in the present report, suggests that plasma MMP-9 levels are increased in patients with AAA.15 The authors identify quantitative increases in MMP-9 in smaller aneurysms compared with that in aortoiliac atherosclerotic occlusive disease and healthy volunteers. Our results confirm these and extend previous observations,3,15,40 demonstrating that both MMP-9 and MMP-3 are elevated in patients with AAA in comparison with healthy volunteers. Pyo and associates41 have suggested that activation of MMP-9 may depend on additional enzymes, such as MMP-3,32 u-PA plasmin,43 and neutrophil elastase.44 This suggests that both MMP-9 and MMP-3 participate in aneurysm development by virtue of their matrix degradation activity.

Elective surgical repair is an effective approach to prevent death from rupture of AAAs.2,16,17 Recently, Hovsepian and associates45 have reported a significant decrease in plasma levels of MMP-9 after surgical exclusion of AAA, suggesting that circulating MMP-9 may provide a biologically relevant marker of successful aneurysm repair. The present study is in agreement with the results, demonstrating that either after OSR or endovascular exclusion by stent graft placement, MMP-9 and MMP-3 decrease similarly during follow-up. Since the source of plasma MMP-9 and MMP-3 levels appears to be the diseased aortic wall, as demonstrated by the immunohistochemical study of aortic biopsies,3,4,14 this implies that both techniques are able to reduce the inflammatory cell infiltrate of the aortic wall.

**MMPs, AAAs, and Endovascular Exclusion**

In recent years, endovascular graft exclusion of AAAs has been proposed as a valid alternative technique in comparison to OSR. In particular, endovascular stent grafts have predictably been proposed as the answer for patients affected by AAA and severe comorbidities that render them unfit for open repair. The avoidance of general anesthetic and major abdominal operation, a shorter duration of hospital stay, a decreased need for intensive care monitoring, and a more rapid recovery favor the endovascular procedure.19,20,46 However, these advantages must be contrasted with the cost of the stent graft47 and the problem of endoleak development.27,48

Endoleak rate ranges from 0% to 47%, depending on the kind of stent graft, patient selection, implantation technique, surgical treatment. Third, an increase in MMP levels after EVG exclusion may help in identifying patients who will have perigraft endoleakage during follow-up with consequent continuous sac pressurization and possible expansion of the aneurysm. Fourth, a decrease in MMP levels during follow-up indicates effective aneurysm exclusion by endovascular treatment.

**Immunohistochemical Analysis**

Immunohistochemical aneurysm biopsy specimens showed evidence of extensive atherosclerosis. Fragmentation and depletion of elastin and smooth muscle cells in the media was noted in all samples. In addition, an inflammatory cell infiltrate, mainly composed of a mixture of macrophages and lymphocytes within the media and the adventitia, was variable. Polymorphonuclear and other inflammatory cells were not prominent. Immunohistochemistry showed an intense positive staining both for MMP-9 and MMP-3 (with mean grading of 3.2 for MMP-9 and 2.4 for MMP-3, respectively, P=NS) within the aortic wall (Figure 3).

**Discussion**

The present study suggests the following conclusions regarding plasma MMP-9 and MMP-3 determination in patients affected by AAA and treated either by EVG exclusion or conventional surgical repair: First, plasma MMP-9 and MMP-3 levels are significantly elevated in patients affected by AAA compared with healthy control subjects. Second, in patients successfully treated by EVG, plasma levels of MMPs during follow-up decrease to a level similar to that after

| Figure 2. Mean (±SEM) MMP-9 (top) and MMP-3 (bottom) plasma value concentrations before and during follow-up after stent graft placement. Plasma levels of both MMP-9 and MMP-3 decreased in patients showing no endoleak (n=23), whereas increase was observed in patients with endoleakage (n=5). In those who were treated for endoleak presence (n=5), significant decrease in MMP-9 and MMP-3 plasma levels compared with pretreatment values was observed 6 months after treatment. | Figure 3. Immunohistochemical study of aortic biopsies,3,4,14 this implies that both techniques are able to reduce the inflammatory cell infiltrate of the aortic wall. |
and aortic morphology. The presence of endoleaks may be associated with further expansion of the aneurysm, which in turn may result in rupture. Therefore it is necessary to closely monitor for endoleaks patients submitted to endovascular repair of AAA by serial CT scan examinations, with a significant increase in costs of the total procedure. Furthermore, the biological significance of endoleaks is not known yet, and CT imaging is not helping in the clinical decision-making, which will require additional treatment. These issues have raised concern that the total cost of endovascular exclusion of AAAs, including the long-term cost for endoleak surveillance, could outweigh the benefits of this treatment strategy when compared with OSR.

Despite the ever-increasing volume of literature detailing elevated expression and protein levels of MMPs in aortic aneurysm, no reports in the literature address the relation between plasma MMP expression and presence of perigraft endoleakage after endovascular treatment of AAA. The most significant finding of this study was that a decrease in plasma MMP levels indicates successful aneurysm exclusion after EVG treatment. We demonstrated that there is a continuous decrease in both MMP-9 and MMP-3 after successful AAA exclusion up to 6 months of follow-up. In addition, an increase in MMP levels after EVG placement represents a reliable marker of ineffective aneurysm exclusion.

Because MMP-9 and MMP-3 plasma levels represent excess enzyme released into the circulation during periods of active matrix catabolism, elevated plasma levels of MMPs may reflect a more active state of degeneration of the aortic wall in the natural history of aneurysm progression. Thus, it will be important to follow patients with different aneurysm sizes to evaluate whether abrupt changes in MMP plasma levels are associated with rapid aneurysm growth, which supports the hypothesis that MMP levels change with aneurysm size. Although one might expect larger aneurysms to be more advanced in their pathological activity, we did not find any correlation between MMP levels and aneurysm size. This result is in agreement with other studies that have shown that size alone does not predict the activity of MMP expression within AAA tissue or even the risk of aneurysm rupture. McMillian and associates have demonstrated that the greatest variability in MMP-9 expression occurs in moderate-sized aneurysms (between 5 and 6.9 cm) compared with small (<4 cm) or large (>7 cm). This correspond to the clinical observation that not all aneurysms expand at the same rate. Indeed, in addition to the long-known notion that size remains the leading determinant of AAA rupture risk, it is possible that changes in the morphology and biology of the arterial wall (reflected by MMP level changes) may also play an important role in determining the risk of rupture in larger aneurysms.

### TABLE 3. Metalloproteinase-9 and Metalloproteinase-3 Baseline and Follow-Up Plasma Levels in Patients Undergoing Surgical or Endovascular Abdominal Aortic Aneurysm Repair

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal</th>
<th>1 mo</th>
<th>3 mos</th>
<th>6 mos</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMP-9 levels, ng/mL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVG (n=30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endoleak yes (n=7)</td>
<td>26.3±12.4</td>
<td>35.4±24.2</td>
<td>40.2±20.9</td>
<td>44.3±20.7</td>
</tr>
<tr>
<td>Endoleak no (n=23)</td>
<td>34.1±22.6</td>
<td>24.5±15.2</td>
<td>23.6±10.4</td>
<td>14.6±7.0</td>
</tr>
<tr>
<td>OSR (n=15)</td>
<td>28±9.9</td>
<td>21.9±12.8</td>
<td>17.8±8.8</td>
<td>14.7±6.6</td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>8.9±2.5</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td><strong>MMP-3 levels, ng/mL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVG (n=30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endoleak yes (n=7)</td>
<td>17.6±9.8</td>
<td>19.6±9.7</td>
<td>19.9±9.8</td>
<td>25±11.5</td>
</tr>
<tr>
<td>Endoleak no (n=23)</td>
<td>18.6±9.8</td>
<td>19.4±8.3</td>
<td>15.4±6.6</td>
<td>10.3±5.4</td>
</tr>
<tr>
<td>OSR (n=15)</td>
<td>26.7±10.8</td>
<td>23.6±9.3</td>
<td>18.9±9.5</td>
<td>12±5.3</td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>8.2±5.3</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Data are reported as mean±SD. Letters indicate P values for paired t test; typographic symbols indicate P values for unpaired t test.

- 2P≤0.05, basal vs 6 months; 2P≤0.05, 1 month vs 6 months; 2P≤0.05, 3 vs 6 months; 2P≤0.05, basal vs 1 month, vs 3 months, vs 6 months; 2P≤0.05, 1 month vs 6 months; 2P≤0.05, 3 vs 6 months; 2P≤0.001, basal vs 3 months; basal vs 6 months; 2P≤0.05, 1 month vs 3 months, 1 month vs 6 months; 2P≤0.05, basal vs 6 months; 2P≤0.001, 1 vs 3 months, vs 6 months; 2P≤0.0001, 3 vs 6 months.
- 2P≤0.001, control vs EVG, vs OSR; 2P≤0.05, OSR vs EVG; 2P≤0.005 Endoleak yes vs Endoleak no; 2P≤0.005 OSR vs Endoleak yes.

MMP-9 levels in EVG patients are shown in the following table.
by guest on August 31, 2017 http://circ.ahajournals.org/ Downloaded from

Figure 3. Abdominal angiography (A) demonstrating AAA not suitable for EVG placement for marked calcifications of aneurysm sac and severe tortuosity of left and right common iliac arteries (B, black arrows). Aortic biopsy obtained at time of OSR showed intense positive staining for MMP-9 (C) and MMP-3 (D) of aortic wall. Note that large thrombus (T) associated with tissue sample is completely negative (C, ×4; D, ×2). Bar in C, 0.20 mm; bar in D, 0.30 mm.

6-month pretreatment values, suggesting complete AAA exclusion at this time.

In the series reported in the present study, 16 of 45 (35.5%) abdominal aortic aneurysms that have been treated were <5 cm in size. Unlike open repair, in which the anatomy of the AAA is not important for the clinical decision to operate, both the feasibility and technical success of EVG repair depend on the anatomic characteristics of the aneurysm. It is well known that smaller aneurysms have better proximal and distal landing zones and straighter access vessels compared with those of larger AAAs. Therefore, EVG repair is expected to be safer and more feasible in the earlier stage of AAA, and some have advocated early intervention with EVG. In addition, the decision to operate on a patient should be made on an individual basis, since all aneurysms are potentially lethal and the rate of expansion and frequencies of rupture are unpredictable. In general, most vascular surgeons suggest repair for all asymptomatic aneurysms >5 cm and for all symptomatic aneurysms. For those measuring 4 to 5 cm in diameter, as the series presented in this study, operation remains controversial. Indeed, although large aneurysms expand more rapidly than small ones, Cronenwett and associates noted that during an average period of 36 months of follow-up, 9% of small aneurysms (mean diameter, 4 cm) ruptured. In addition, data from a Markov decision model analysis suggested that operation was preferred to watchful waiting for aneurysms 4 to 5 cm in diameter. On the other hand, the recent UK randomized trial showed no benefit of open repair over ultrasound surveillance in AAAs ranging between 4 and 5.5 cm in diameter. The surgical mortality rate in this study was 5.8%. However, it is noteworthy that 60% of the patients assigned to the surveillance group ultimately underwent surgery during the study period of 6 years, mainly because of aneurysm enlargement beyond 5.5 cm. In addition, 7% of patients in the surveillance group died of aneurysmal rupture during the surveillance period, thus suggesting that factors other than size may be important in aneurysm growth and rupture.

Clinical Implications

The present study supports the potential utility of plasma MMP-9 and MMP-3 determination as specific biomarkers for AAA disease. Because plasma MMPs determination by ELISA is a simple and readily available technique, it could be useful to use such markers in clinical studies for assessing the response to medical, surgical, or interventional treatment instead of monitoring patients with multiple CT scan examinations. The reappearance of these enzymes in the peripheral circulation can signal the development of a continuous sac pressurization and recurrent aneurysm. Larger studies will obviously be required to confirm these possibilities.

Conclusions

Population-based screening studies have revealed that >10% of individuals harbor an unsuspected AAA. Although the majority remain asymptomatic, the natural history is characterized by progressive increase in diameter and eventual rupture. Recognition that different MMPs participate in the pathobiology of AAAs has fostered intensive research aimed to use these enzymes as therapeutic targets in this disease. The present study may provide new clinical evidence in support of this concept and suggests that a simple MMP plasma level determination could be used to monitor the success of different therapeutic strategies for the treatment of AAAs.

Acknowledgments

Dr Sangiorgi and Dr Inglese are grateful to Gianfranco Mantella for support in the purchase of metalloproteinase ELISA kits. The authors wish also to thank Franco Di Giorgio for expert technical assistance.

References


Plasma Levels of Metalloproteinases-3 and -9 as Markers of Successful Abdominal Aortic Aneurysm Exclusion After Endovascular Graft Treatment

Giuseppe Sangiorgi, Rita D'Averio, Alessandro Mauriello, Monya Bondio, Marina Pontillo, Serenella Castelvecchio, Santi Trimarchi, Valerio Tolva, Giovanni Nano, Vincenzo Rampoldi, Luigi Giusto Spagnoli and Luigi Inglese

doi: 10.1161/hc37t1.094596

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/suppl_1/I-288