Aminoterminal Propeptide of Type III Procollagen and Matrix Metalloproteinases-2 and -9 Failed to Serve as Serum Markers for Abdominal Aortic Aneurysm

T. Eugster,* A. Huber, T. Obeid, I. Schwegler, L. Güke and P. Stierli

1University Vascular Center Aarau/Basel, and 2Department of Laboratory Medicine, Kantonsspital Aarau, Aarau, Switzerland

Objectives. Matrix-metalloproteinase (MMP)-2 and -9 and aminoterminal propeptide of type III collagen (NIIINP) have been reported to be elevated in patients with abdominal aortic aneurysm (AAA). The aim of our study was to test NIIINP, MMP-2 and -9 as potential serum markers for AAA in a large population group at risk for AAA.

Methods. Fifty-five to 70 year old men were screened for AAA by abdominal ultrasound. Simultaneously, blood samples were taken and the patients were interviewed for known risk factors for AAA. Patients with a dilatation of the infrarenal aorta of ≥25 mm (Group 1, n=76) were compared to randomly assigned patients with normal aortic diameters (Group 2, n=83). A third group consisted of patients scheduled for operation of AAA (n=19).

Results. A total of 987 men were investigated with ultrasound. Seventy-six (7.7%) had an aortic dilatation ≥25 mm. Aortic dilatation was correlated with age (P=0.0001). However, serum levels of NIIINP and MMP-2 were not different between the three groups of patients. For MMP-9 there was a weak inverse correlation with lower serum levels in patients with aortic dilatation (P=0.043).

Conclusions. Both MMP-2 and -9 and NIIINP failed to show relevance as serum markers for aortic dilatation. Our results are, therefore, in contradiction to previous published results. AAAs cannot be diagnosed with a simple blood test.

Keywords: Abdominal aortic aneurysm; Serum marker; Metalloproteinases.

Introduction

Ruptured abdominal aortic aneurysm (AAA) is associated with a high rate of fatality and is an important cause of sudden death.1,2 Early detection through screening has been the only available way of detecting the majority of AAA in the community.3–9 Degradation of extracellular matrix proteins in the aortic wall is a key event in the formation of AAA, resulting in decreased elastin concentration and increased collagen turnover. Several studies have shown the presence and involvement of matrix metalloproteinases (MMP) and aminoterminal propeptide of type III procollagen (NIIINP) in AAA disease. Theoretically, highly expressed soluble MMP or NIIINP could be continuously released into the systemic circulation and be measurable in the blood of patients with AAA. Preliminary studies have suggested this concept.10–15 The purpose of our study was to determine the validity of MMP-2 and -9 and NIIINP as serum markers for AAA in a large cohort at risk for AAA.

Patients and Methods

We were invited to join a large prospective study initiated by our colleagues from urology. The study invites all male inhabitants at the age of 55–70 years from our county (i.e. 45,000) to undergo a prospective control for prostatic cancer. During 1999–2001, 1300 men were randomly assigned by postal code and invited by letter to screen for AAA. One thousand and forty-one (80%) agreed. Thirty-two men (3%) had exclusion criteria (i.e. surgery during the last 6 months, liver disease, cancer or rheumatoid arthritis). Twenty-two did not show up for ultrasound despite repetitive invitation and were excluded. Finally, 987 men were enrolled in the study. All participants gave written informed consent. The study was approved by
the local ethical committee and the procedures were in accordance with institutional guidelines.

The diameter of the infrarenal aorta was measured by ultrasound (Panther 2002 Advanced Diagnostic Imaging, B-K Medical, Germany) in an anterior–posterior direction by three investigators. The largest diameter was chosen. The first 20 examinations of each investigator were cross-checked by an experienced radiologist to control for accuracy. Twenty milliliters of blood was obtained from the cubital vein of each participant and stored at $-25^\circ \text{C}$. All participants were screened with a standardized questionnaire for smoking (current or past 5 years), hypertension, diabetes mellitus and first-degree relatives with known aneurysmal disease. Group 1 consisted of patients with aortic dilatation $\geq$25 mm. A control group of 83 men without aortic dilatation comprised Group 2. These men were chosen at random (frequent given name) from the remaining 911 men without aortic dilation $<25$ mm. Group 3 ($n = 19$) consisted of patients with known AAA that were operated on during study recruitment.

Serum levels of MMP-2 and -9 and PIIINP were determined in all three groups. MMP-2 and -9 were batch-analyzed with use of a commercially available enzyme-linked immunosorbent assay (ELISA) kit specific for human MMP-2 and -9 (Amersham Biosciences Europe GmbH, Freiburg, Germany). All samples were analyzed in duplicate and averaged. If the MMP sample levels from a patient varied by $>$10%, the serum was retested in triplicate and the averaged value was taken. The within-assay precision ($<6\%$) and the between-assay precision ($<10\%$) were determined by the manufacturer. PIIINP values were determined with a standardized radioimmuno-assay test (Orion Diagnostica, Espoo, Finland) in the same fashion.

Data were analyzed by STATA (Stata Corporation, College Station, TX, USA). All data are presented as mean $\pm$ SD. The Wilcoxon’s rank test was used to compare two groups with measured values and the Kruskal–Wallis test was used to compare more than two groups. Rank correlation according to Spearman was used to assess dependent variables. Statistical significance was set at $P < .05$.

## Results

### Age and aortic diameter

Aortic dilatation $\geq 25$ mm was present in 76 (7.7%) of the 987 men. A diameter of 5 cm or more was found in five (0.5%) patients. The mean age of all men was 62.1 ($\pm 4.3$) years. The mean age of men with aortic dilatation $\geq 25$ mm was 63.6 ($\pm 4.1$) years. Age was distributed normally within age groups (55–59, 60–64 and 65–70 years). Aortic diameter was correlated to patients age ($P = .0001$, Table 1).

### Serum values and aortic diameter

Mean values for NIIINP were $322\pm130$ µg/l (range 27–919), $312\pm101$ µg/l (range 143–748) and $334\pm113$ µg/l (range 104–552) in Groups 1, 2 and 3, respectively. Differences between groups were not statistically significant (Table 2, Fig. 1). MMP-2 mean values were 1473 $\pm 287$ (range 880–2320), 1468 $\pm 343$ (152–2640) and 1432 $\pm 328$ (980–2140) ng/ml in Groups 1, 2 and 3, respectively. There was no statistical difference between the three groups (Table 2, Fig. 2). For MMP-9 mean values were as follows: Group 1 371 $\pm 284$ (range 108–2044) ng/ml, Group 2 455 $\pm 499$ (102–4480) ng/ml and Group 3 281 $\pm 125$ (108–502) ng/ml. These differences showed a weak statistical significance between the three groups ($P = 0.043$).

### Table 1. Comparison of patients divided into age groups and dilatation of the aorta at 25 shows normal distribution between groups

<table>
<thead>
<tr>
<th>Age</th>
<th>Aorta $\geq 25$ mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>55–59 years ($n = 332$)</td>
<td>$n = 16$ (4.8%)</td>
</tr>
<tr>
<td>60–64 years ($n = 345$)</td>
<td>$n = 25$ (7.2%)</td>
</tr>
<tr>
<td>65–70 years ($n = 310$)</td>
<td>$n = 35$ (11.3%)</td>
</tr>
<tr>
<td>All ($n = 987$)</td>
<td>$n = 76$ (7.6%)</td>
</tr>
</tbody>
</table>

Expansion of aortic diameter with increasing age is significant ($P = .0001$).

Fig. 1. Distribution of serum values of NIIINP in relation to aortic diameter (mm). $P = .378$ for comparison of all three groups (Kruskal–Wallis test). Aorta, aortic diameter; s values of Group 1 (patients with aortic dilatation $\geq 25$ mm, $n = 76$); k values of Group 2 (randomized population without aortic dilatation, $n = 83$); g2 values of Group 3 (patients operated on for AAA, $n = 19$).

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Table 2. Regression analysis was negative, i.e. MMP-9 values for aortas with higher diameter were lower (Table 2, Fig. 3).

Discussion

Different histopathologic studies have demonstrated that aneurysmal tissue is characterized by elevation in elastolytic and collagenolytic activity. Satta et al. found elevated serum levels for NIIINP in patients with AAA compared to patients with peripheral arterial occlusive disease (PAOD). In a follow-up study over 3 years the values for NIIINP increased according to enlarging aortic diameter. In our study, we found no correlation of higher serum levels of NIIINP and a larger diameter of the infrarenal aorta. A possible explanation for the disparity in the findings may be that the control group in Satta’s study were patients with PAOD. Our control group consisted of a randomized population of 911 men of the same age as the patients with aortic dilatation. In another study, Lindholt and coworkers were not able to show a correlation between dimension of AAA and NIIINP blood levels. In a follow-up study with elastin and collagen markers, NIIINP was not useful in predicting expansion of the AAA to a size requiring surgery. In accordance with our results and the discussed studies we conclude that NIIINP does not seem to be a serum marker for AAA.

In 1991, Vine described the important role of metalloproteinases in degenerative aortic disease. This paper was followed by publications dealing with the behavior of matrix metalloproteinases in the wall of the infrarenal aorta and its influence on smooth muscle cells. All these investigations were performed with aneurysmatic aortic wall tissue. McMillan et al. reported a relationship between the diameter of the AAA and MMP-9 values. They found the highest amount of MMP-9 in the wall of aortic aneurysms with a diameter between 5 and 6.9 cm. They concluded that MMP-9 may be responsible for the dilatation of the AAAs over 5 cm. As in previous studies, this study included only a small number of patients (n = 19).

Freestone et al. were able to show that MMP-2 was the dominating enzyme in the wall of small early aneurysms. Later, with increasing aortic diameter and predominant inflammatory reaction in the aortic wall, MMP-9 became the leading elastolytic enzyme. In a follow-up study, McMillan and Pearce reported elevated plasma levels of MMP-9 in patients with

Table 2. Comparison of values of NIIINP, MMP 2 and 9 between different groups of patients (SD standard deviation of the mean)

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 76)</th>
<th>Group 2 (n = 83)</th>
<th>Group 3 (n = 19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIIINP (mean, SD)</td>
<td>322 (±130) µg/l</td>
<td>312 (±101) µg/l</td>
<td>334 (±113) µg/l</td>
<td>.378</td>
</tr>
<tr>
<td>MMP 2 (mean, SD)</td>
<td>1473 (±287) ng/ml</td>
<td>1468 (±342) ng/ml</td>
<td>1432 (±328) ng/ml</td>
<td>.716</td>
</tr>
<tr>
<td>MMP 9 (mean, SD)</td>
<td>371 (±284) ng/ml</td>
<td>455 (±499) ng/ml</td>
<td>281 (±125) ng/ml</td>
<td>.043</td>
</tr>
</tbody>
</table>

Group 1, patients with aortic dilatation ≥ 25 mm; Group 2, population without aortic dilatation; Group 3, patients operated on for AAA.

Fig. 2. Distribution of serum values of MMP-2 in relation to aortic diameter. P = .716 for comparison of all three groups (Kruskal–Wallis test). Aorta, aortic diameter; s, values of Group 1 (patients with aortic dilatation ≥ 25 mm, n = 76); k, values of Group 2 (randomized population without aortic dilatation, n = 83); g, values of Group 3 (patients operated on for AAA, n = 19).

Fig. 3. Distribution of serum values of MMP-9 in relation to aortic diameter (mm). P = .043 for comparison of all three groups (Kruskal–Wallis test). The y-axis scale is logarithmic. Aorta, aortic diameter; s, values of Group 1 (patients with aortic dilatation ≥ 25 mm, n = 76); k, values of Group 2 (randomized population without aortic dilatation, n = 83); g, values of Group 3 (patients operated on for AAA, n = 19).
AAA compared to patients with PAOD and to healthy control patients. Patients with AAA, however, were significant older (mean age 72.7 years) than patients with PAOD (mean age 60.5 years) and members of the healthy control group (mean age 35.3 years). An influence of age can, therefore, not be excluded. Hovsepian et al. described elevated plasma levels for MMP-9 in patients with AAA. After surgical correction of the AAA MMP-9 values returned to normal. Several investigators described decreasing levels of MMP-9 and possibly MMP-2 after operation of an AAA. After endovascular aneurysm the values seem to decrease faster than after open fixation of an AAA.

Our results do not support these previous investigations. In our study, we found no correlation between serum levels of MMP-2 and -9 and the diameter of the AAA. There are some shortcomings to our study. A possible explanation for the disparate results is the long duration of the recruitment procedure. In order to achieve a higher study power, some 1000 men were screened for aortic aneurysm. The advantage of this action was to achieve an ideal representative population for AAA. The disadvantage consisted of the time needed (i.e. more than 2 years) to recruit and screen all men included. Therefore, we believe that the time-factor was not relevant.

A sound explanation for elevated MMP-9 levels is suggested by Lindholt et al. They reported a large difference between median serum and plasma values for MMP-9, possibly due to release of these proteases and their protease inhibitors upon platelet activation.

In conclusion, we found no predictive value of NIIINP, MMP-2 and -9 as serum markers for AAA. Our results are in contradiction to previous studies. Further studies with platelet-free plasma are still necessary to prove the value of NIIINP, MMP-2 and -9 as circulating markers for AAA.

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