View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Eur J Vasc Endovasc Surg **20**, 281–285 (2000)
doi:10.1053/ejvs.2000.1151, available online at http://www.idealibrary.com on IDEAL®

The Plasma Level of Matrix Metalloproteinase 9 may Predict the Natural History of Small Abdominal Aortic Aneurysms. A Preliminary Study*

J. S. Lindholt†[1], S. Vammen[1], H. Fasting[1], E. W. Henneberg[1] and L. Heickendorff[2]

[1]*Department of Vascular Surgery, Viborg Hospital, Denmark, and* [2]*Department of Clinical Biochemistry, Aarhus Amtssygehus, Aarhus University Hospital, Denmark*

Objectives: *increased levels of various proteinases have been detected in abdominal aortic aneurysms (AAA) and are assumed to cause the degradation of the aortic wall. To determine whether systemic measurement of these proteinases and their inhibitors may predict the natural cause of AAA.*
Methods and material: *serum (S) and plasma (P) samples were obtained from 121 men following the diagnosis of a small AAA (3–5 cm) at population screening. Annual control scans were performed to check for expansion. Circulating levels of elastase-alpha$_1$antitrypsin-complexes, alpha$_1$antitrypsin, matrix metalloproteinase (MMP) 2 & 9, tissue-inhibitor-matrixproteinase 1 & 2, procollagen III-N-terminal-propeptide, and elastin-peptides were measured in a random group of 36 men.*
Results: *alpha$_1$antitrypsin was significantly and positively associated with expansion. Similarly, P-MMP9 levels were significantly associated with size and expansion. There was a difference between median serum and plasma values, probably because of secretion from platelets.*
Conclusion: *P-MMP9 and P-alpha$_1$antitrypsin may predict the natural history of AAA.*

Key Words: Abdominal aortic aneurysm; Natural history; Expansion; Metalloproteinase; Elastin peptides.

Introduction

Previous reports have described a 0.0–0.5% annual rupture rate of abdominal aortic aneurysms (AAA) below 5 cm in diameter.[1–7] AAA above 5 cm found at autopsy have an increased risk of rupture with increasing AAA size; 20–50% risk for AAA 5–7 cm in diameter, and a 95% risk for those above 10 cm.[1,8,9] These autopsy reports have been confirmed in clinical studies.[3,5–7,10–13] The indication to operate is therefore determined by the size of the AAA. However, small AAA rupture occasionally, while some operated AAA would never have ruptured. Furthermore, increasing use of ultrasonographic scanning and CT-scanning has increased the number of small AAA that are diagnosed. Some will expand to a size which demands operation, but the patients will then be older, with increased surgical risk, or may have developed a contraindication for operation. Finally, conservative treatment of such small AAA is reported to produce psychological stress.[14,15] The results from the U.K. Small Aneurysm Trial[15] seem to confirm these problems. In order to make further improvements, development of additional predictive tools other than size alone are needed.

The activity and perhaps insufficient inhibition of proteases may cause degradation of the aortic matrix and subsequent remodelling of elastin and collagen that leads to aneurysmal formation. Increased levels of elastase and two matrix metalloproteinases (MMP2 and MMP9) have been detected in AAA. Consequently, systemic measurements of these proteases and their inhibitors may predict the natural history of aneurysms and allow better selection of patients, i.e. for surgery or conservative management.

Material and Methods

In 1994, 4404 males aged 65–73 years were invited to participate in a randomised screening trial for AAA at their regional hospital.[16–18] B-mode scans were carried out with a Phillips SDR 1550, using a linear 4 MHz transducer and calliper light pen.

* The manuscript was first presented at the 13th annual meeting in the European Society of Vascular and Endovascular Surgery in Copenhagen 1999.
† Please address all correspondence to: Dr. J. S. Lindholt, Ph.D., Laerkevej 11, 8900 Randers, Denmark.

1078–5884/00/090281+05 $35.00/0 © 2000 Harcourt Publishers Ltd.

An AAA was defined by an aortic diameter of 30 mm or more. AAAs of 5 cm or more were referred to a vascular surgeon. AAAs of 3–4.9 cm were offered yearly follow-up examinations to check for expansion. Blood samples were taken, and serum samples were left for coagulation for 45 min. Plasma was obtained by using EDTA as an anticoagulant and platelet free plasma was prepared. Serum (S) and plasma (P) samples were then frozen at −20 °C.

Initially, and at the following annual control scans, two observers and the same scanner were used. The observers used a standardised method for measuring the maximal anteroposterior diameter. Validation of the measurements between the two observers is very acceptable.[19] We have also reported earlier that the expansion was more linear than exponential;[16] consequently the mean annual expansion rate was calculated as: ((Present AP-diameter – Initial AP-diameter)/days of observation) 365.25 days. The observers were blinded to the results from the blood samples.

Of the 4404 men who were invited, 3344 (76%) attended screening, and of them 141 (4.2%) had AAA. Of these, 19 (13.5%) were more than 5 cm. The remaining 122 patients with a small AAA were offered annual scans. Of these, four (3.3%) died the following year, one (0.8%) was operated on as an emergency because of symptoms of aneurysmal rupture, and seven (5.7%) did not attend for control. At control, two (1.8%) proved to be false positives. Thus, 108 men with a small AAA had their AAA controlled at a minimum once. A random sample of 36 cases was sampled after 4 years by the randomisation facility in Epiinfo version 6.02. No differences between the selected and the unselected cases were noticed concerning age and initial AAA size. Concentrations of the following were determined: S-alpha$_1$antitrypsin, S- and P-elastase-alpha$_1$antitrypsin-complexes, S- and P-matrix metalloproteinase 2 (MMP2) and P-MMP9, S- and P-tissue-inhibitor-matrixproteinases 1 and 2 (TIMP1 and 2), and S-elastin-peptides,[20] and S-Procollagen III-N-terminal propeptide (PIIINP).[21,22] MMP2, TIMP1, and TIMP2 were measured using enzyme-linked-immunosorbent assays (ELISA) from Amersham Biotech (Biotrak, Buckinghamshire, U.K.), while MMP9 was determined with a kit from R&D Systems (Oxon, U.K.). Between assay coefficients of variances in percentages were <10% for all assays. The methods for elastin-peptides and elastase-alpha$_1$antitrypsin-complexes have been reported earlier.[20,23]

Initial aneurysm size and mean annual expansion rate were compared between those below and those above the median concentrations of the various assays with Wilcoxon's rank sum tests.

The trial was approved by the local scientific ethics committee and reported to the Central Control of Registers.

Results

Neither the plasma nor the serum levels of elastase-complexes were predictors of AAA size or expansion. However, their major inhibitor, alpha$_1$antitrypsin, was significantly and positively associated with expansion (Table 1), though the association did not increase by summation (S-elastase-complexes + S-alpha$_1$antitrypsin).

Concerning the MMP metabolism, P-MMP9 levels were significantly associated with size and expansion, while MMP2, their common inhibitor, and the ratio (total MMP/total TIMP) did not predict size or expansion.

A large difference between median serum and plasma values was observed, probably due to release of these proteases and their protease inhibitors upon platelets activation. The finding indicates that further research could be restricted to platelet-free plasma. Consequently, MMP9 analyses were not performed on serum (Table 2).

The systemic level of S-elastin peptides was still predictive of aneurysmal expansion,[20] while the level of PIIINP-propeptides was not associated to expansion. Neither the "Matrix Sum", defined as S-elastin peptides + S-PIIINP, or the "Matrix Ratio", defined as S-elastin peptides/S-NPIIIP, added further predictive potentials (Table 3).

Thus, three variables were associated with expansion. Their non-parametric (Spearman) correlation coefficients are listed in Table 3.

Discussion

The sample size was chosen because of the sizes of kits, and because calculations during the design showed that the only two known strong predictors of the natural history of AAA (S-elastin peptides and initial AAA-size) would have been significantly associated with expansion if the sample size and tests were used as in this study, and their association with expansion and AAA size was the same as in one of our earlier reports.[20] Consequently, we believe that potential predictors of the natural history of AAA among the tested variables would be detectable in the present study.

Table 1. The association of serum and plasma levels of elastase and alpha$_1$antitrypsin with aneurysmal size and expansion.

		Initial AAA size (mm)			AAA expansion (mm/year)			
Serological marker	Median mg/l	Below median	Above median	*p*	Below median	Above median	*p*	Correlation coefficient r
S-elastase complexes	13.0	35.6	36.3	0.98	2.91	2.50	0.47	
P-elastase-complexes	6.0	33.8	37.9	0.11	1.82	3.47	0.24	
P-alpha$_1$-antitrypsin	1390	34.7	37.1	0.32	1.80	3.49	0.05	0.42 (0.08–0.67)
Total-P-alpha$_1$-antitrypsin	1400	34.8	36.9	0.58	2.00	3.42	0.05	0.42 (0.08–0.67)
Alpha$_1$anti-trypsin ratio	0.0046	35.4	36.3	0.92	2.54	2.88	0.85	

Total-P-alpha$_1$antitrypsin = P-alpha$_1$antitrypsin + P-elastase-complexes.
Alpha$_1$-antitrypsin-ratio = P-elastase-complexes/P-alpha$_1$antitrypsin.
p: *p*-value of Wilcoxon's rank sum test comparing the groups below and above the median value.
r: Spearman's correlation coefficient (95% confidence limits in parentheses).

Table 2. The association of serum and plasma levels of matrix metalloproteinases (MMP) and tissue-inhibitor-matrixproteinases (TIMP) with aneurysmal size and expansion.

		Initial AAA-size (mm)			AAA-expansion (mm/year)			
Serological marker	Median ng/ml	Below median	Above median	*p*	Below median	Above median	*p*	Correlation coefficient r
P-MMP9	60	33.7	38.8	0.04	0.74	3.30	0.01	0.33 (0.01–0.53)
S-MMP2	575	36.5	35.8	0.94	2.17	2.00	0.95	
P-MMP2	135	35.8	36.1	0.93	2.78	2.60	0.71	
S-TIMP1	865	35.5	36.6	0.69	2.68	1.44	0.11	
P-TIMP1	336	34.0	37.9	0.10	2.14	3.25	0.48	
S-TIMP2	115	38.1	34.5	0.12	3.30	2.26	0.70	
P-ratio	1.00	35.6	37.2	0.64	2.72	2.67	0.96	

p-ratio = (P-MMP2 + P-MMP9)/P-TIMP1.
p: *p*-value of Wilcoxon's rank sum test comparing the groups below and above the median value.
r: Spearman's correlation coefficient (95% confidence limits in parentheses).

Table 3. The association of serum and plasma levels of elastin peptides and procollagen III-N-terminal propeptides (PIIINP) with aneurysmal size and expansion.

		Initial AAA-size (mm)			AAA-expansion (mm/year)			
Serological marker	Median mg/l	Below median	Above median	*p*	Below median	Above median	*p*	Correlation coefficient r
S-elastin peptides	330	35.9	36.2	0.90	1.05	3.43	0.01	0.51 (0.20–0.73)
S-PIIINP	320	33.4	36.6	0.61	2.71	1.44	0.18	
Matrix sum	640	34.9	37.3	0.31	1.83	3.71	0.04	0.26 (−0.10–.56)
Matrix ratio	1.03	35.5	36.3	0.75	2.26	3.16	0.53	

Matrix sum = S-elastin peptides + NPIIIP.
Matrix ratio = S-elastin peptides/NPIIIP.
p: *p*-value of Wilcoxon's rank sum test comparing the groups below and above the median value.
r: Spearman's correlation coefficient (95% confidence limits in parentheses).

Due to the large number of tested variables, and in order to limit the chance of random significance, the statistical tests were kept to a minimum, and only supported with correlation coefficients in significant findings.

Elastase in blood is immediately inactivated by anti-proteinases.[23] Consequently, these complexes were measured instead of elastase activity. Increased levels of elastase have been noticed in AAA walls,[24–27] but the complexes did not predict aneurysmal expansion in this study. This discrepancy between the two observations may be due to the fact that elastase is an acute phase-reactant reacting on inflammation, and signs of an inflammatory response in so-called non-inflammatory AAA have been reported.[28] Furthermore, the previously observed fourfold increased levels of elastase in walls of ruptured AAA[29] may be due to the inflammatory response caused by the

retroperitoneal bleeding.[23] Finally, the potential aortic derived elastase complexes may be too small in the systemic circulation compared to complexes from other organs, e.g. the lungs, where the complexes are strongly and negatively correlated to pulmonary function.[30] On the other hand, the levels of the major systemic elastase inhibitor, alpha$_1$antitrypsine, was positively associated with expansion. This may be caused by smoking.[30–32] Nevertheless, a predictive value is possible, but a larger sample size is needed for clarification and determination of the potential clinical recommendations.

Increased levels of MMP2 have been detected, particularly in small AAA,[33–38] but the systemic levels could not predict expansion. This may be due to similar reasons as found for the elastase-complexes. However, P-MMP9 was strongly associated with expansion. Whether this is a pathogenetic observation remains unknown, but P-MMP9 seems to be a potential predictor of the natural history of AAA. A larger sample size is again needed for evaluation of the clinical implications of this observation.

Elastin is a major component of the human abdominal aorta. In AAA, the structure and amount of elastin are altered, and the measurement of circulating elastin peptides has been suggested as a method for monitoring pathological processes involving degradation of elastic tissue, as in emphysema[39–42] and AAA.[20] After 4 years of follow up, S-elastin peptides continue to have a potential strong predictive value.

The role of the aortic collagen metabolism is controversial. Signs of increased degradation have been reported in histological studies,[43,44] while signs of increased regeneration (PIIINP) have also been reported.[45] Unfortunately, it is not possible to detect specific degradation products from the collagen III metabolism, and measurement of common collagen degradation products such as proline and hydroxyproline seems irrational because it would be dominated by degradation products from the bone metabolism. Consequently, it is only possible to evaluate the regenerative part of the collagen III metabolism and its relative amount compared with elastin peptides. None of these two variables seem to have any predictive value.

Conclusion

In conclusion, only P-MMP9, S-elastin peptides and P-alpha$_1$antitrypsin were associated with initial aneurysm size and annual expansion. In this preliminary study, the associations of P-MMP9 and P-alpha$_1$antitrypsin seemed almost as strong as with S-elastin-peptides, the most powerful serological predictor of aneurysmal expansion known. However, a larger sample size is needed to evaluate the potential clinical recommendations.

Acknowledgements

The Danish Heart Foundation, The Foundation of Asta and Rosa Jensen, and the Health Department of Viborg County are thanked for financial support. Dr. Per Jaeger, Skive Sygehus, Dr. Sten Antonsen, University of Odense, and the project's nurse Anette K. Sahlholdt are thanked for committed practical assistance. Finally, the excellent technical assistance of Kirsten Hald is gratefully acknowledged.

References

1 Sterpetti AV, Cavallaro A, Cavallari N *et al.* Factors infuencing the rupture of abdominal aortic aneurysms. *Surg Gyn & Obst* 1991; **173**: 175–178.
2 Brown PM. The selective management of small abdominal aortic aneurysms: The Kingston Study. *J Vasc Surg* 1992; **15**: 21–27.
3 Nevitt MP, Ballard DJ, Hallett JW. Prognosis of abdominal aortic aneurysms. A population-based study. *N Engl J Med* 1989; **321**: 1009–1103.
4 Greenhaulgh RM, Fowkes FGR, Powell JT *et al.* When should small asymptomatic aneurysms be operated upon? In: Greenhaulgh RM, ed. *The Causes and Management of Aneurysms.* London: Saunders, 1990; 457–460.
5 Johansson G. Survival in patients with abdominal aortic aneurysms; Comparison between operative and nonoperative management. *Eur J Vasc Surg* 1990; **4**: 497–502.
6 Scott RAP, Wilson NM, Ashton HA *et al.* Is surgery necessary for abdominal aortic aneurysm less than 6 cm im diameter? *Lancet* 1993; **342**: 1395–1396.
7 Glimåker H, Holmberg L, Elvin A *et al.* Natural history of patients with abdominal aortic aneurysm. *Eur J Vasc Surg* 1991; **5**: 125–130.
8 Darling RC. Ruptured arteriosclerotic abdominal aortic aneurysms. A pathological and clinical study. *Am J Surg* 1970; **119**: 397–401.
9 Darling RC, Messina CR, Brewster DC, Ottinger LW. Autopsy study of unoperated abdominal aortic aneuryms. The case for early resection. *Cardiovasc Surg* 1976; **56**: 161.
10 Flanigan DP. Selective management of high risk patients with an abdominal aortic aneurysm. *Surg Gyn & Obst* 1980; **150**: 171–176.
11 Quriel K. An evalation of new methods of expressing aortic size: relationship to rupture. *J Vasc Surg* 1992; **4**: 497–502.
12 Szilagyi DE, Elliott JP, Smith RF. Clinical fate of the patients with asymptomatic abdominal aortic aneurysm unfit for surgical treatment. *Arch Surg* 1972; **104**: 600–606.
13 Perko MJ, Schroeder TV, Olsen PS *et al.* Natural history of abdominal aortic aneurysm: a survey of 63 patients treated nonoperatively. *Ann Vasc Surg* 1993; **7**: 113–116.
14 Lindholt JS, Vammen S, Henneberg EW *et al.* Psychological consequences of screening for abdominal aortic aneurysms and surveillance of small abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 2000; **20**: 79–83.
15 UK Small Aneurysm Trial Participants. Health service costs and quality of life for early elective surgery or ultrasonographic

surveillance for small abdominal aortic aneurysms. *Lancet* 1998; **352**: 1656–1660.
16 Lindholt JS. Considerations and experiences of screening for abdominal aortic aneurysms (Ph.D. Thesis). Copenhagen: FADL's Forlag, 1998.
17 Lindholt JS, Henneberg EW, Fasting H *et al.* High-risk or mass screening for abdominal aortic aneurysms. *Br J Surg* 1997; **84**: 40–42.
18 Lindholt JS, Juul S, Fasting H *et al.* Is screening for abdominal aortic aneurysm acceptable for the population? Selection and recruitment for hospitalbased screening for abdominal aortic aneurysm. *J Public Health Med* 1998; **20**: 211–217.
19 Lindholt JS, Vammen S, Juul S *et al.* The validity of ultrasonographic scanning as screening method for abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg* 1999; **17**: 472–475.
20 Lindholt J, Heickendorff L, Fasting H *et al.* Serum-elastin-peptides as predictor for the expansion of small abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 1997; **14**: 12–16.
21 Jensen LT. The aminoterminal propeptide of type III procollagen. Studies on physiology and pathophysiology (thesis). Copenhagen: Lægeforeningens forlag, 1996.
22 Risteli J, Niemi S, Trivedi P *et al.* Rapid equilibrium radioimmunoassay for the aminoterminal propeptide of type III procollagen. *Clin Chemistry* 1988; **34**: 715–718.
23 Antonsen S, Wanscher M. An ELISA for elastase A1-protease inhibitor complexes in human plasma and serum. *Scand J Clin Lab Invest* 1993; **53**: 145–153.
24 Sans M, Moragas A. Mathematical morphologic analysis of the aortic medial structure. Biomechanical implications. *Analytical and Quantitative Cytology and Histology* 1993; **15**: 93–100.
25 Baxter BT, Davis VA, Minion DJ *et al.* Abdominal aortic aneurysms are associated with altered matrix proteins of the non-aneurysmal aortic segments. *J Vasc Surg* 1994; **19**: 797–802.
26 Brophy CM, Reilly JM, Smith GJW *et al.* The Role of Inflammatory Infiltrate in Non-specific Abdominal Aortic Aneurysm disease. *Ann Vasc Surg* 1991; **5**: 229–233.
27 Cohen JR. Altered aortic protease and antiprotease activity in patients with ruptured abdominal aortic aneurysm. *Surg Gyn & Obst* 1987; **164**: 355–357.
28 Rijbroek A, Moll FL, Dijk HA *et al.* Inflammation of the abdominal aortic aneurysm wall. *Eur J Vasc Surg* 1994; **8**: 41–46.
29 Busuttil RM. Collagenase activity of the human aorta. *Arch Surg* 1980; **115**: 1373–1378.
30 Lindholt JS, Heickendorff L, Antonsen S *et al.* The natural history of abdominal aortic aneurysms with and without chronical obstructive pulmonary disease. *J Vasc Surg* 1998; **28**: 226–232.
31 Janoff A. Elastases and emphysema. *Am Rev Respir Dis* 1985; **132**: 417–433.
32 Wright RR. Elastic tissue of normal and emphysematous lungs. A three-dimensional study. *Am J Pathol* 1961; **39**: 355–363.
33 Freestone T, Turner RJ, Coady A *et al.* Inflammation and matrix metalloproteinases in the enlarging abdominal aortic aneurysm. *Arteriosclerosis, Thrombosis & Vascular Biology* 1995; **15**: 1145–1151.
34 Newman KM, Jean-Claude J, Li H *et al.* Cellular localisation of matrix metalloproteinases in the abdominal aortic aneurysmal wall. *J Vasc Surg* 1994; **20**: 814–820.
35 Patel MI, Melrose J, Ghosh P *et al.* Increased synthesis of matrix metalloproteinases by aortic smooth muscle cells is implicated in the etiopathogenesis of abdominal aortic aneurysms. *J Vasc Surg* 1996; **24**: 82–92.
36 Sakalihasan N, Delvenne P, Nusgens BV *et al.* Activated forms of MMP2 and MMP9 in abdominal aortic aneurysms. *J Vasc Surg* 1996; **24**: 127–133.
37 Rosen SE, Baruch L, Fisher EA *et al.* Current status of echocardiographic automated border detection. *Imaging Advances* 1994.
38 Petersen E, Geneitis A, Ängqvist KA. Activity of matrix metalloproteinases 2 and 9 in abdominal aortic aneurysms. An explanation for rupture or continuous growth? *ESVS98, Paris* 1998 (Abstract).
39 Kucich U, Christner P, Lippmann M *et al.* Immunologic measurement of elastin derived peptides in human serum. *Am Rev Respir Dis* 1983; **127**: 28–30.
40 Darnule TV, McKee M, Darnule AT *et al.* Solid-phase radioimmunoassay for estimation of elastin peptides in human sera. *Analyt Biochem* 1982; **122**: 302–307.
41 Dillon TJ, Walsh RL, Scicchitano R *et al.* Plasma elastin-derived levels in normal adults, children and emphysematous subjects. *Am Rev Respir Dis* 1992; **146**: 1143–1148.
42 Schriver EE, Davidson JM, Sutcliffe MC *et al.* Comparison of elastin peptides cocentrations in body fluids from healthy volunteers, smokers and patients with chronical obstructive pulmonary disease. *Am Rev Respir Dis* 1992; **145**: 762–766.
43 Halloran BG, Davis VA, McManus BM *et al.* Localization of aortic disease is associated with intrinsic differences in aortic structure. *Journal of Surgical Research* 1995; **59**: 17–22.
44 Thornell LE. Familial abdominal aortic aneurysm. On the distribution of elastin collagen I and III, and the intermediate filament proteins desmin and vinmentin. In: Norrgaard Ö, ed. *Familial Occurrence of Abdominal Aortic Aneurysm*. Umeå: Umeå University Medical Dissertations, 1985; 78–103.
45 Satta J, Juvonen T, Haukipuro K *et al.* 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**: 155–160.

Accepted 4 April 2000