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Five-year Results of Elastin and Collagen Markers as Predictive Tools in the Management of Small Abdominal Aortic Aneurysms*

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Objective: *small abdominal aortic aneurysms (AAAs) do rupture and only half of AAAs above 5 cm would have ruptured unoperated. Furthermore, conservative treatment of AAAs may cause psychological side effects and impaired quality of life. To optimise the indication and time for operation for AAAs, we analysed whether serum elastin peptides (EP), procollagen-IIIN-terminal propeptide (PIIINP), and the initial AAA size could predict operation for AAAs in initially conservatively treated AAA.*

Material and methods: *in 1994, 4404 65–73 year old males were invited to hospital-based screening for AAAs by ultrasonography. Seventy-six percent attended. One hundred and forty-one (4.2%) had AAAs (def: +30 mm). Nineteen were offered operation (AAA +50 mm), and 112 were followed with annual control scans for 1–5 years (mean 2.5 years). Of these, 99 had their EP (ng/ml) and PIIINP (ng/ml) determined using ELISA and RIA techniques. Two observers and one scanner were used.*

Results: *the mean expansion rate was 2.7 mm/year. The initial AAA size (*r*=0.46; 0.26–0.61), EP (*r*=0.31; 0.11–0.49), and NPIIIP (*r*=0.24; 0.02–0.44) was independently significant associated to expansion rate in a multiple linear regression analysis including the three mentioned variables. The multivariate formula could by ROC curve analysis predict cases reaching 5 cm in diameter within 5 years with a sensitivity and specificity of 91% and 87%, respectively, increasing to 91% and 94%, respectively, by accepting a 2 mm variation in those measurements. Twenty-three were lost to follow up, 21 of these due to death or severe illness. Of these, seven would have been predicted to reach an AAA size recommendable for surgery. If all 23 were included in the analysis, the sensitivity and specificity would have been 87% and 85%, respectively.*

Conclusion: *a predictive model using EP, PIIINP, and initial AAA size seems capable of predicting nine out of 10 AAAs that will be operated on within 5 years. However, a larger sample size is needed for clinical recommendations.*

Key Words: Abdominal aortic aneurysm; Natural history; Expansion; Mass screening; Surveillance; Elastin peptides; Surgery.

Introduction

Previous reports have described an 0.0–0.5% annual rupture rate of abdominal aortic aneurysms (AAAs) below 5 cm in diameter,[1–7] while AAAs above 5 cm found at autopsy have increased frequency of rupture with increasing AAA size; from 20–50% of AAAs with 57 cm in diameter to 95% in AAAs above 10 cm in diameter.[1,8,9] These autopsy reports have been confirmed by clinical studies.[3,5–7,10–13] Therefore the indication of operation is mainly determined by the size of the AAA. However, small AAAs do occasionally rupture, while some AAAs operated on would never have ruptured.[1–13] Furthermore, increasing use of ultrasonographic scanning and CT scanning have increased the number of small AAAs diagnosed. A number of these will expand to a size demanding operation, but the patients will then be older with increased risk at operation or will eventually have developed a contraindication for operation. Finally, conservative treatment of small AAAs has been shown to cause psychological stress.[14,15] These problems may increase, if screening for AAAs proves to be beneficial.[15] Therefore, a more specific indication for operation would be desirable.

Elastin and collagen are the major matrix components of the human abdominal aorta. In AAAs the structure and the amount of both matrix proteins is changed.[16] Increased levels of elastase in aneurysmal walls,[16–19] and increased systemic levels of procollagen-III-N-terminal propeptide (PIIINP) have been reported in aneurysmal cases.[20] Finally, we have previously reported preliminary results suggesting serum elastin

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peptide (EP) levels could be a possible strong predictor of the expansion of small AAAs, even after adjustment for AAA size.[21]

After 5 years of screening and surveillance of AAAs, we have analysed further whether EP alone, or in combination with PIIINP and initial AAA size, could be a potential clinical useful predictor for operation for AAAs in initially conservatively treated AAAs.

Material and Methods

As a part of a randomised screening trial assessing the cost effectiveness of screening for AAAs, 4404 65–73 year-old males were invited to B-mode-ultrasonographic screening for AAA at their regional hospital in 1994. Their mean age was 68.5 years (SD: 2.68). Of these, 3344 (76%) attended and were examined by a doctor and a nurse, specially trained in ultrasonography, who alternated between organising and registering the patients and performing the scans. An AAA was defined as an anterior–posterior 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.[22]

All patients with an AAA consulted the trial doctor for information, interview, examination, and a rescan. Blood samples were taken within 10 days of the initial scan. The sampling was performed by the nurse and trial doctor in order to handle each sample in a standardised way. Serum was stored at -21 °C until analysis.

One hundred and forty-one men had an AAA diagnosed at screening (4.2%), 19 were above 5 cm at the diagnosis and were referred for surgery; the remaining 87% cases were offered annual control scans. Of these, 112 cases have now been followed for 1–5 (mean 2.5) years. Of these, data including blood samples were complete in 99 cases (88%).

Expansion was calculated as the change in the anterior–posterior diameter during the whole observation period transformed to annual units. It was in mean 2.7 mm/year (SD: 2.1).

The inter-observer variation (2 SD) of the infrarenal anterior-posterior aortic diameter was 1.7 mm.[23] The concentration of elastin-peptides (EP) in serum was determined using an inhibition type of ELISA performed essentially as described earlier[21] and initially by Giro and Davidson.[24] The intra- and inter-assay coefficients of variation were 6 and 10%, respectively. Standards and serum samples were analysed in duplicates. The trial was approved by the local scientific ethics committee and reported to the central control of Registers.

SPSS 10.0 was used for Pearson's correlation analysis, multiple linear regression analyses, and elaboration of ROC curves. For analyses of the ROC curves the null hypothesis was that the test had a performance similar to the diagonal line, i.e. the area under the curve was 0.5. If the lowest 95% confidence limit for the area under the curve was above 0.5, a significant predictive test was said to be present.

Results

After 2 and 5 years, 16 and 24 AAAs, respectively, had expanded to more than 5 cm and were referred for preoperative evaluation. The mean expansion rate was 2.7 mm/year. Initial AAA size ($r = 0.46$; 0.26–0.61), EP ($r = 0.31$; 0.11–0.49), and NPIIIP ($r = 0.24$; 0.02–0.44) was significantly correlated to mean annual expansion rate (Table 1).

All three variables continued to be significantly associated with expansion in a multivariate linear regression analysis using the three variables as independent variables and expansion rate as the dependent variable. The formula of the predicted expansion rate was:

$$\text{Predicted expansion rate (mm/year)} = 0.1731(\text{initial AAA size(mm)}) + 0.0088(\text{EP(Ng/ml)}) + 0.3579(\text{PIIINP(Ng/ml)})6.968.$$

The ROC curves for predicting operation within 2 years of surveillance concerning EP, PIIINP, initial AAA size, and the multivariate model showed that the variables were statistically significant predictive for expansion to a size recommendable for surgery, except PIIINP. The multivariate model reached a sensitivity and specificity of 94% and 87%, respectively, while the sensitivity and specificity concerning initial AAA size was 93% and 90%, respectively. Within the first 2 years, nine were lost for follow-up. Seven of these were due to development of severe illness. None of their AAAs were predicted to become 5 cm or more within 2 years. The corresponding ROC curves for 2 years, excluding those lost for follow-up within the first 2 years, showed again that all the variables were statistically significantly predictive for expansion to a size recommendable for surgery, except PIIINP. The predictive value of the multivariate model was unaffected, maintaining a sensitivity and specificity of 94% and 87%, respectively, while the sensitivity and

Table 1. Correlation matrix concerning the variables used in the prediction of abdominal aortic aneurysms expanding to sizes recommendable for surgery. Pearson's correlation analysis (*r*). Confidence intervals listed in parentheses.

	Expansion rate (mm/year)	Initial AAA size (mm)	S-elastin peptides (ng/ml)	S-PIIINP (ng/ml)
Expansion rate (mm/year)		0.48 (0.29–0.63)	0.33 (0.12–0.51)	0.24 (0.01–0.44)
Initial AAA size (mm)	0.48 (0.29–0.63)		0.24 (0.00–0.44)	0.09 (−0.14–0.30)
S-elastin peptides (ng/ml)	0.33 (0.12–0.51)	0.24 (0.01–0.44)		0.01 (−0.21–0.23)
S-PIIINP (g/ml)	0.24 (0.01–0.44)	0.09 (−0.14–0.30)	0.01 (−0.21–0.23)	

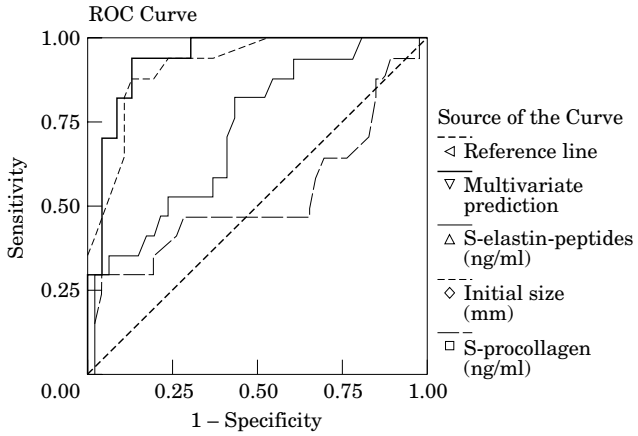

Fig. 1. ROC curves for predicting operation within 2 years of surveillance concerning EP, PIIINP, initial AAA size, and the multivariate model are shown concerning those followed for 2 years excluding those lost for follow up within the first 2 years.

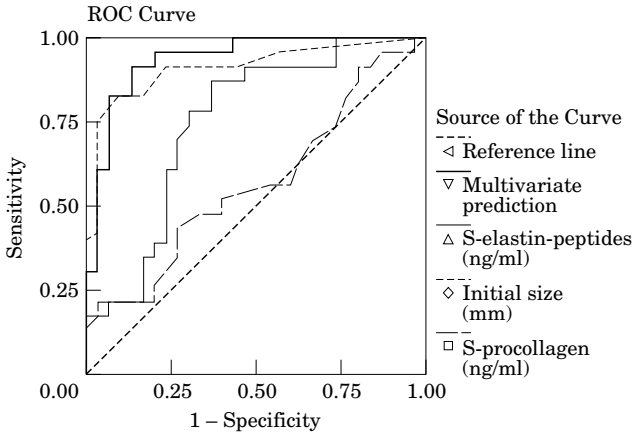

Fig. 2. ROC curves for predicting operation within 5 years of surveillance concerning EP, PIIINP, initial AAA size, and the multivariate model are shown concerning those followed for 5 years excluding those lost for follow up within the first 5 years.

specificity concerning initial AAA size decreased to 83% and 87%, respectively (Fig. 1).

The ROC curves for predicting operation within 5 years of surveillance concerning EP, PIIINP, initial AAA size, and the multivariate model showed again that all variables were statistically significant predictive for expansion to a size recommendable for surgery except PIIINP. The best predictive variable was the multivariate model, reaching a sensitivity and specificity of 87% and 85%, while the sensitivity and specificity concerning initial AAA size was 82% and 79%, respectively.

Within the first 5 years, 23 were lost for follow-up. Twenty-one of these were due to development of severe illness. Seven of their AAA would have been predicted to become 5 cm or more within 5 years. The ROC curves for predicting operation within 5 years of surveillance concerning EP, PIIINP, initial AAA size, and the multivariate model concerning those followed for 5 years, excluding those lost for follow-up within the first 5 years showed again that all the variables were statistically significantly predictive for expansion to a size recommendable for surgery expect PIIINP.

The best predictive variable was the multivariate model, reaching a sensitivity and specificity of 91% and 87%, respectively (Fig. 2), increasing to 91% and 94%, respectively, by accepting 2 mm of variation of the measurements. The sensitivity and specificity concerning initial AAA size was both 83%, increasing to 89% and 91%, respectively, by accepting 2 mm of variation of the measurements (Table 2).

Discussion

The studied cohort were recruited from a randomised screening study which addressed the costs and effectiveness of screening. If any, the potential benefits seem five to six times greater in males compared to females. Consequently, only males were studied.

A retrospective study of 56 cases with AAA or

Table 2. Initial AAA size and multivariate model for predicting small abdominal aortic aneurysms expanding to 5 cm or more within two or five years without and with acceptance of 2 mm variation of the measurements.

Model	Years	±2 mm	Sensitivity	Specificity	Predictive value of a positive test	Predictive value of a negative test
Initial AAA size	2	−	83% (59–96)	87% (74–95)	71% (48–89)	93% (81–99)
		+	85% (55–98)	97% (89–99)	85% (55–98)	97% (89–99)
Multivariate	2	−	94% (55–98)	87% (74–95)	73% (50–89)	98% (87–99)
		+	92% (64–99)	98% (91–99)	92% (94–99)	91% (91–99)
Initial AAA size	5	−	83% (61–95)	83% (65–84)	79% (58–93)	86% (68–96)
		+	89% (71–98)	91% (77–98)	89% (71–98)	91% (77–98)
Multivariate	5	−	91% (72–99)	87% (69–96)	84% (64–96)	93% (77–99)
		+	91% (70–99)	94% (81–99)	91% (70–99)	94% (81–99)

thoracic aortic aneurysm found that exponential models of expansion had a slightly better fit than linear models.[25] We have not been able to show better predictive values by exponential models of growth rate in this cohort. The explanation could be due to the relatively small AAA followed, the low interobserver variation, or the lack of "rapid expansion" as indication for operation. A high interobserver variation eventually combined with rapid expansion as an indication for surgery tends to a falsely exponential growth pattern just before referral for surgery.

We wanted to develop a simple model involving the endpoints in the aortic degradation. The role of chronic obstructive pulmonary disease and smoking in aneurysmal progression and rupture must be through the measured elastin and collagen metabolism. However, potential mechanical factors such as blood viscosity and blood pressure were not included. We do not have viscosity data, and the role of hypertension is debatable. It is a risk factor for having an AAA but blood pressure did not correlate with expansion in our cohort as in most other studies.[21,22,26] Non-inflammatory and inflammatory AAA may behave differently; however, we did not find any with clinical or ultrasonographic signs of inflammatory aneurysms and none of the aneurysms referred for surgery showed retroperitoneal fibrosis. Nevertheless, we assessed whether CRP correlated with expansion, PIIINP or elastin peptides. No significant correlations were noticed.

A combination of markers of the elastin and collagen matrix metabolism with the initial AAA size seem logical because the prediction must depend on how close the AAA is from its goal (operation indication), and the degree of the elastin degradation and the compensatory collagen anabolism[16] because structural changes in these two matrix components are the main components in the weakness of the aneurysmal wall.

Our results suggest a potential clinical useful predictive tool based upon the metabolism of elastin and collagen combined with the initial AAA size in decision-making in the management of small AAAs on the basis of sensitivity and specificity of 91% and 87%, respectively, increasing to 91% and 94%, respectively, by accepting 2 mm of interobserver variation of the measurements (Table 2).

It seems relevant to question whether the relatively small increase in the predictive value compared to the predictive values of only using the initial AAA size worth the effort and cost needed is to measure the additional two variables. However, our observed interobserver variation of 1.68 mm seems smaller than most other studies,[23,27–31] and furthermore the same scanner was used to make all the measurements. Consequently, the predictive value of the initial AAA size could be overestimated compared with a situation where the predictive model is generalised. The multivariate model would be less sensitive for a higher interobserver variation concerning the diameter. Furthermore, by including elastin and collagen markers the most metabolic active AAAs are predicted to have the highest expansion rate. Such small AAAs may have the highest risk of rupturing while they are still small. Finally, the costs of the serological markers are lower than £10 per patient.

It also seems relevant to question whether the prediction that 8–9 out of 10 AAAs that will be operated on within 5 years is sufficiently satisfactory, because 10–15% of the recommendable operations would have to be carried out on patients with AAAs that would not reach 5 cm in diameter within 5 years or would be lost for follow-up mainly due to death or severe illness. However, a larger amount of those lost for follow-up because of severe illness or death may have a high frequency of contraindications for surgery. Furthermore, the risk of rupture is removed, their quality of life normalised,[14,15] and the operation could probably be performed at lower risk because of a lower age. Finally, the application of a predictive tool may reduce the psychological consequences concerning those that are not predicted recommendable for surgery. In all, we find the application of the tested predictive tool

relevant. However, the numbers are relatively small with consequently relatively large confidence intervals. Furthermore, the operation is potentially lethal (4–7%), and the risk of rupture is limited in small AAAs (0.25–0.5% annually) but only a relatively small amount of additional operations (approx 10%) would be needed. Carrying out the operations earlier could reduce the operative mortality and morbidity more than the additional procedures will produce, and would thus reduce the overall physical, psychological, and economical costs. In all, the model needs to be validated in a prospective and possibly randomised study involving a larger number of AAAs, both sexes, and several observers and scanners in order to analyse the cost-effectiveness of using the model before it can be clinically recommended. The model suggests 10% more operations, but if the operations are carried out earlier when patients are more fit complications and operative mortality may be reduced by more than 10%.

Finally, the consequences of the use of the potentially varying distribution of various peptides and polyclonal antibodies as ingredients in the ELISA must be evaluated. The ELISA is performed by coating microtitre plates with human aortic elastin. These purified human aortic elastin peptides are made by elastin fragmentation with hot oxalic acid. The other part of the ELISA is specific animal antihuman elastin. IgG was raised against alpha elastin prepared from human aorta.[32] Consequently, the ELISA measures a wide range of peptides that depend on the initial elastin fragmentation and the following humoral response. Consequently, future production of further ingredients may vary considerably. The consequences of this variation concerning the predictive value of the natural history of AAAs is unknown but are due to be examined.

Conclusion

A combination of s-elastin-peptides, PIIINP, and the initial AAA size seems to be a potentially useful tool in decision-making in the management of small AAAs with a high sensitivity and specificity (both about 90%). However, a larger sample size is needed, and the consequences of the use of the potentially varying distribution of elastin peptides and antibodies as ingredients in the ELISA must be evaluated before clinical application can be recommended.

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