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Differential Protein Expression in Serum of Abdominal Aortic Aneurysm Patients — A Proteomic Approach

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Abstract *Objective:* The aim of the study is to investigate the differential expression of proteins in serum of abdominal aortic aneurysm (AAA) patients in relation to aneurysm size (D_{max}) and progression.
Methods: Two-dimensional differential in-gel electrophoresis (2D-DIGE) together with tandem mass spectrometry (MS/MS) was used to analyse the serum proteome from patients with small (D_{max} 30–54 mm) AAA, either stable (increase D_{max} <5 mm year^{-1}; $n = 8$) or progressive (increase D_{max} ≥5 mm year^{-1}; $n = 8$), and large (D_{max} ≥55 mm; $n = 8$) AAA. The identified proteins were quantitatively validated in a larger population ($n = 80$).
Results: Several proteins were differentially expressed in serum of small stable, small progressive and large AAA. Three validated proteins (immunoglobulin G (IgG), α1-antitrypsin (α1-AT) and Factor XII activity) showed strong correlation with D_{max}. Size combined with either Factor XII activity or α1-antitrypsin had minimal effect on the prognostic value in predicting aneurysm progression compared with size alone (area under the curve (AUC), 0.85; 95% confidence interval (CI), 0.73–0.97; $p < 0.001$ and AUC, 0.85; 95% CI, 0.72–0.98; $p < 0.001$ vs. AUC, 0.83; 95% CI, 0.71–0.96; $p < 0.001$, respectively).
Conclusion: The present study indicates that both Factor XII and α1-antitrypsin are found in increased amounts in the serum of patients with expanding AAA. However, combination of either Factor XII or α1-antitrypsin with aneurysm diameter had little effect on prediction of aneurysm progression versus diameter alone.

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Abdominal aortic aneurysm (AAA) is a degenerative disease of the abdominal aorta leading to progressive dilatation, intraluminal thrombus (ILT) formation and rupture. Survival associated with AAA rupture is extremely low, with rates ranging from 10% to 15%, while elective surgery has an overall survival rate of 95%. It is widely recognised that the risk of rupture increases with increasing diameter, with the threshold for elective repair at ≥55 mm diameter (D_{max}). However, a significant number of AAA ≥55 mm never experience rupture,[1] while small AAA can rupture unexpectedly.[2] Early detection and elective AAA repair is, for those at risk of rupture, a critical step to limit mortality associated with aneurysm rupture.[3] Better approaches are needed for follow-up of patients with small asymptomatic AAA. Most patients with small AAA are prospectively followed by serial imaging until elective repair is indicated,[3–7] although growth rate differs substantially between individuals.[8] Besides identifying patients at risk for AAA progression[9,10] and rupture,[11] new biomarkers may also help to elucidate the molecular mechanisms behind this disease and eventually generate treatments to reduce progression rate.

Proteomics provides powerful opportunities to examine protein expression patterns of tissues or body fluids.[12–15] Compared with conventional two-dimensional (2D) gel electrophoresis, 2D differential in-gel electrophoresis (2D-DIGE) enables quantitative comparison of protein expression and reduces the analytical variability through the use of an internal standard.[16]

The aim of the present study was to detect differential serum protein expression related to aneurysm size and progression. To do so, we used 2D-DIGE together with matrix-assisted laser desorption ionisation time-of-flight tandem mass spectrometry (MALDI-TOF/TOF) to detect and identify differentially expressed proteins between small stable, small progressive and large AAA. Differential expression of the identified proteins was quantitatively validated using routine immunochemical techniques.

Materials and Methods

Sample collection

Between January 2006 and January 2009, blood samples (e.g., serum, ethylene diamine tetraacetic acid (EDTA) and citrate plasma) were obtained from patients with small asymptomatic AAA, who were invited to participate in a standardised 6-monthly follow-up study. Furthermore, preoperative blood samples were obtained from patients undergoing elective or emergency reconstruction of the infrarenal abdominal aorta at the Department of Vascular Surgery, Maastricht University Medical Centre+ (MUMC+, The Netherlands). The Medical Ethical Committee approved the research protocol and all participants gave written informed consent. The present study enrolled 48 consecutive patients with small (D_{max} 30–54 mm) asymptomatic AAA, either stable (increase $D_{max} < 5$ mm year^{-1}; $n = 27$) or progressive (increase $D_{max} \geq 5$ mm year^{-1}; $n = 21$), and 32 consecutive AAA patients undergoing elective open or endovascular repair ($D_{max} \geq 55$ mm). Patients with symptomatic, mycotic or ruptured AAA were excluded. Demographics of the study subjects at baseline were obtained by interview and examination (Table 1). These included sex, age, smoking habit (current smoker, ex-smoker since 1 year or never smoked), hypertension (use of anti-hypertensive drugs), family history, coronary disease (history of unstable angina pectoris, myocardial infarction, coronary

Table 1 Baseline demographics of patients with stable, progressive and large AAA.

	Stable AAA ($n = 27$)	Progressive AAA ($n = 21$)	Large AAA ($n = 32$)	ANOVA p-value
Male sex	21 (77.8%)[a]	15 (71.4%)[b]	31 (96.9%)	0.029
Age (years) Range	69.9 ± 6.3[c] (61–83)	71.7 ± 8.2 (54–82)	74.4 ± 6.0 (63–87)	0.040
AAA diameter (mm) Range	36.6 ± 4.2[d] (31–43)	44.9 ± 6.5[d] (30–53)	72.1 ± 10.1 (55–92)	<0.001
Family history	6 (22.2%)	1 (4.8%)	7 (21.9%)	N.S.
Hypertension	21 (77.8%)	15 (71.4%)	19 (59.4%)	N.S.
Smoking history[e]				
Never	1 (3.7%)	3 (14.3%)	3 (11.5%)	N.S.
Quitted	18 (66.7%)	8 (38.1%)	14 (53.9%)	
Current	8 (29.6%)	10 (47.6%)	9 (34.6%)	
Coronary disease	12 (44.4%)	11 (52.4%)	16 (50.0%)	N.S.
COPD	2 (7.4%)	2 (9.5%)	5 (15.6%)	N.S.
PAD	13 (48.1%)	6 (28.6%)	5 (15.6%)	0.030
Chronic renal failure	3 (11.1%)	2 (9.5%)	3 (9.4%)	N.S.
Statin use	18 (66.7%)	14 (66.7%)	17 (53.1%)	N.S.

N.S. not significant different.
[a] Significantly different compared to large AAA: $p = 0.03$.
[b] Significantly different compared to large AAA: $p = 0.01$.
[c] Significantly different compared to large AAA: $p = 0.04$.
[d] Significantly different compared to large AAA: $p < 0.001$.
[e] Smoking history is available for 26 patients with large AAA.

artery bypass grafting or percutaneous transluminal coronary angioplasty), chronic obstructive pulmonary disease (COPD; based on the Global initiative for chronic Obstructive Lung disease (GOLD) criteria),[17] peripheral arterial disease (PAD; ankle/brachial pressure index <0.9), chronic renal failure (glomerular filtration rate <15 ml min^{-1} · 1.73 m^{-2}) and statin use.

Blood was allowed to coagulate at room temperature for 30 min and centrifuged at 4000*g* for 15 min at 4 °C. Exactly 1 h after blood collection, samples were stored in aliquots at −80 °C pending analysis. Maximal anterior−posterior D_{max} was measured by one observer, with intra-observer variability of 2 mm (data not shown), using ultrasound for follow-up patients and computed tomography angiography before surgery.

Sample preparation

For 2D-DIGE, serum samples with no freeze−thaw cycle were selected from patients with either small stable ($n = 8$), small progressive ($n = 8$) or large ($n = 8$) AAA. Samples from time point t were used to discover factors prospectively predicting aneurysmal progression in 1 year. Aneurysmal progression was calculated as the change in D_{max} from time point t to $t+12$ months.

To improve the DIGE analysis, individual serum samples were fractionated based on their biophysical properties using the Expression Difference Mapping Kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Six fractions were obtained per patient; fractions pH3, pH4 and pH5 were pooled together (denoted fraction A), and fractions pH7 and pH9 were mixed (denoted fraction B). The organic fraction was discarded, as isoelectric focussing was carried out on strips with a pH range of 3−10. The pH of the fractions was adjusted to 8.5 by 50mM NaOH to obtain optimal labelling conditions.

2-D DIGE

Fractions A and B were separately analysed, comparing protein expression differences between the groups within each fraction. Fractions from individual participants were randomly labelled with Cy3 or Cy5, according to the manufacturer's instructions (GE Healthcare, Uppsala, Sweden). Dye swapping was performed to control for preferential labelling by one of the dyes. The labelling reaction was quenched on ice in the dark for 10 min by scavenging non-bound dye with 10 mM lysine (Sigma−Aldrich, St. Louis, MO, USA). Three labelled samples, two analytical and a Cy2-labelled internal standard were pooled and an equal volume of 2 × lysis buffer (7M urea, 2M thiourea, 4% CHAPS buffer, 0.04% bromophenol blue, 2% dithiothreitol (DTT), 2% IPG-buffer pH 3−10) was added.

Samples were focussed on an IPGphor using immobilised DryStrip gels (3−10 pH range, non-linear, 18 cm), which were passively pre-rehydrated for 6 h with DeStreak Rehydration Solution supplemented with 0.5% IPG-buffer. Strips were equilibrated for 15 min with gentle shaking in equilibration solution (6M urea, 2% sodium dodecylsulphate (SDS), 50mM Tris pH 8.8, 0.02% bromophenol blue, 30% glycerol) supplemented with 1% DTT, followed by 2.5% iodoacetamide in fresh equilibration solution for an additional 15 min of incubation with gentle shaking. Second-dimension sodium dodecylsulphate−polyacrylamide gel electrophoresis (SDS−PAGE) was carried out on polyacrylamide gels (12.5% T, 3% C) using Ettan DALTtwelve. Gels were run at 20 °C, 0.5 W gel^{-1} for 1 h and 15 W gel^{-1} until the bromophenol blue frontier reached the bottom of the gel and subsequently scanned on the Ettan DIGE imager using CyDye-specific excitation/emission wavelengths. Gels were analysed using DeCyder 7.0 software (GE Healthcare). Differentially expressed protein spots present in >70% of the gels were selected for identification using MS/MS.

Protein identification and in-gel digestion

Preparative gels, loaded with 300 μg unlabelled protein, were run using previous conditions. Gels were fixed for 30 min in 10% CH_3OH and 7% CH_3COOH followed by an overnight incubation in Sypro Ruby (Bio-Rad). Thereafter, gels were again fixed in 10% CH_3OH and 7% CH_3COOH for 45 min followed by a thorough rinse with milliQ. Protein spots of interest were picked from the Sypro-stained gel using the automated Ettan Spot Picker (GE Healthcare) into 96-well plates and in-gel digestion was carried out on the MassPREP digestion robot (Waters, Manchester, UK).

Gel plugs were destained twice in 100mM NH_4HCO_3, 50% (v/v) CH_3CN for 10 min, then dehydrated in 100% CH_3CN for 5 min, after which supernatant was removed, and gel plugs were allowed to air dry for 10 min. Cysteines were reduced with 10mM DTT in 100mM NH_4HCO_3 for 30 min followed by alkylation with 55mM iodoacetamide in 100mM NH_4HCO_3 for 20 min. Gel plugs were washed with 100mM NH_4HCO_3 and subsequently dehydrated with 100% CH_3CN. Trypsin suspended in 50mM NH_4HCO_3 was added (12 ng μl^{-1}) and allowed to digest at 40 °C for 5 h. Peptides were extracted twice with 1% (v/v) formic acid, 2% (v/v) CH_3CN.

MS/MS and database analysis

Peptide solutions were mixed at a 1:1 ratio with 5 mg ml^{-1} α-cyano-4-hydroxycinnamic acid (CHCA) matrix in 50% CH_3CN, 0.1% trifluoroacetic acid (TFA) and spotted in duplo. Spots were allowed to air dry for homogeneous crystallisation. Spectra were acquired in positive ion reflectron mode on a 4800 MALDI-TOF/TOF analyser (Applied Biosystems, Foster City, CA, USA). Tandem MS fragmentation spectra were acquired for each sample, averaging 500 laser shots per fragmentation spectrum on each of the eight most abundant ions present in each sample. The generated peak list was searched with the MASCOT search engine by GPS Explorer v.3.9. Software (Applied Biosystems) against the Swiss-Prot protein database with taxonomy at *Homo sapiens*; trypsin and keratin peaks were excluded. One mis-cleavage was tolerated; carbamidomethylation was set as a fixed modification and oxidation of methionine as an optional modification. Protein charge was set at 1+, mass tolerance for precursor ion at 150 ppm and MS/MS tolerance at 0.2 Da. No restrictions were made on protein mass. Identification was

considered confident when total ion and protein score were above the 95% confidence interval (CI).

Laboratory validation measurements

Quantitative validation measurements were carried out using native serum or citrate plasma from consecutive patients with stable ($n = 27$), progressive ($n = 21$) and large ($n = 32$) AAA. Serum concentrations of alpha-1 antitrypsin (α1-AT) and immunoglobulin G (IgG) were analysed on the BN ProSpec (Siemens Healthcare Diagnostics, The Hague, The Netherlands). Analyses were performed at the Department of Clinical Chemistry, MUMC+ with following reference values: α1-AT (0.75–1.85 g l^{-1}) and IgG (4.5–14.0 g l^{-1}). Citrate plasma Factor XII activity and D-dimer concentrations were analysed on the Sysmex CA-7000 (Siemens Healthcare Diagnostics) at the Department of Haematology, MUMC+ with following reference values: 60–140% and <500 ng ml^{-1}, respectively.

D-dimer was not found as one of the discriminating proteins using DIGE, but was included as a 'positive control' measurement testing the validity of our population, as recent literature has evidenced a clear relationship with AAA presence, diameter and progression.[18–21]

Statistical analysis

Data were analysed with Predictive Analytics Software (PASW) Statistics 18.0 Software (IBM Corporation, Somers, NY, USA). Categorical variables, expressed as percentages, were compared using the Pearson chi-squared test. Analysis of variance (ANOVA) was used to compare continuous demographic variables, expressed as mean ± standard deviation, applying the Bonferroni correction for pair-wise comparison. Associations between biomarker concentrations and aneurysm diameter were calculated with linear regression. Multivariate linear regression was used to correct the associations for possible bias due age, sex and PAD. One-way ANOVA with Bonferroni *post hoc* testing (DeCyder 7.0 software, GE Healthcare) was used to detect significant differences in protein abundances between the three experimental groups.

Results

Identification of differentially expressed proteins in serum from AAA patients

On average, approximately 700 spots were detected on each gel. In total, 26 spots were differentially expressed between patients with stable and large AAA; with two up-regulated and nine down-regulated spots in fraction A and three up-regulated and 12 down-regulated spots in fraction B of patients with stable AAA. Fraction A as well as B showed one up-regulated spot in patients with large AAA compared with patients with progressive AAA. Only fraction B revealed significant differential expression between progressive and stable AAA, with eight spots up-regulated in stable AAA.

All spot features of interest were trypsin digested and submitted to MS/MS for identification. We were able to identify seven spots representing five unique proteins that were significantly differentially expressed between the experimental groups (Fig. 1 and Table 2).

Validation of differentially expressed proteins in serum from AAA patients

To confirm the 2D-DIGE differential protein expression, quantitative validation of the results was carried out on serum or citrate plasma using a larger sample set including the samples analysed with DIGE ($n = 80$). Although the difference in Ig spot abundance between large and stable AAA was minimal, significantly higher serum IgG concentrations were found in stable and progressive AAA compared with large AAA (18.4 ± 4.8 and 17.5 ± 4.9 vs. 8.9 ± 2.6 g l^{-1}, $p < 0.001$, Fig. 2A). Serum α1-AT concentrations were also increased in patients with stable and progressive AAA relative to patients with large AAA (1.62 ± 0.25 and 1.62 ± 0.37 vs. 1.16 ± 0.25 g l^{-1}, $p < 0.001$, Fig. 2B). Notably, serum IgG concentrations were elevated above the upper reference limit in almost all small AAA and were positively correlated ($r = 0.67$; $p < 0.001$) with serum α1-AT concentrations. Factor XII was down-regulated in stable AAA, as indicated by the DIGE

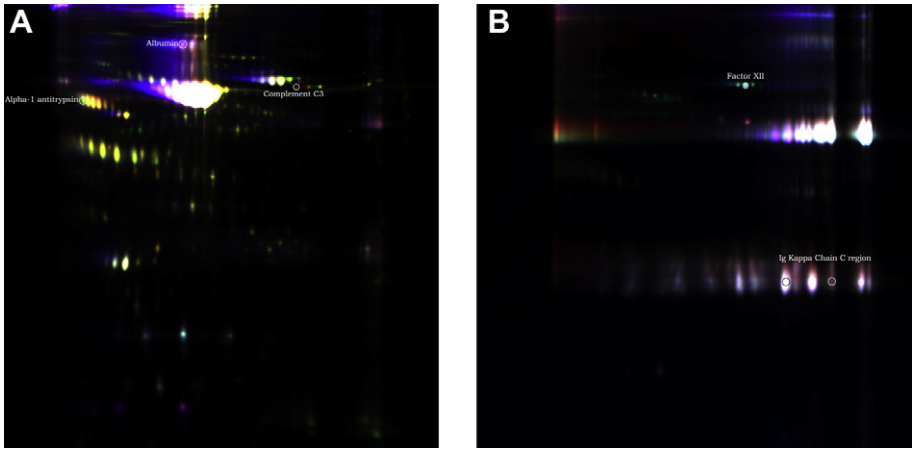

Figure 1 Proteome map of the differentially expressed and identified proteins in fraction A and fraction B.

Table 2 Differentially expressed proteins in serum of stable, progressive and large AAA identified by MALDI-TOF/TOF after 2D-DIGE analysis.

Swiss-Prot ID	Name	Total Ion Score (95% CI)[a]	Stable AAA	Progressive AAA	Large AAA	ANOVA	Stable vs large AAA	Stable vs progressive AAA	Progressive vs large AAA
			Standardized abundance: mean ± SD			*p*-value	*p*-value	*p*-value	*p*-value
Fraction A									
P02768	Albumin	401 (100%)	1.27 ± 0.36	1.98 ± 0.80	2.54 ± 0.62	0.009	0.007	–	–
P01024	Complement C3	215 (100%)	1.29 ± 0.20	1.01 ± 0.35	1.93 ± 1.12	0.022	–	–	0.020
P01009	α-1 antitrypsin	341 (100%)	1.89 ± 0.75	1.14 ± 0.28	1.03 ± 0.51	0.026	0.025	–	–
Fraction B									
P00748	Factor XII	126 (100%)	0.09 ± 0.04	0.36 ± 0.36	0.99 ± 0.59	<0.001	<0.001	–	–
P01834	Ig κ chain C region	65 (100%)	1.06 ± 0.10	0.98 ± 0.15	0.88 ± 0.08	0.043	0.032	–	–
P01834	Ig κ chain C region	39 (99.95%)	1.06 ± 0.17	0.87 ± 0.10	0.87 ± 0.12	0.025	0.042	0.044	–
P01834	Ig κ chain C region	309 (100%)	1.17 ± 0.18	0.95 ± 0.14	0.97 ± 0.11	0.021	–	0.027	–

[a] This score describes the confidence of the identification and proteins with a confidence interval percentage above 95% were considered confidently identified.

analysis. Interestingly, Factor XII activity was significantly lower in plasma of large AAA compared with stable and progressive AAA (53.6 ± 34.9 vs. 104.5 ± 36.3 and 97.5 ± 24.9%, $p < 0.001$, Fig. 2C). In almost all AAA patients, plasma D-dimer concentrations were elevated above the reference limit and were significantly different between patients with stable, progressive and large AAA (Fig. 2D).

Aneurysm size was significantly correlated with serum IgG ($r = -0.65$; $p < 0.001$), serum α1-AT ($r = -0.55$; $p < 0.001$), plasma D-dimer ($r = 0.62$; $p < 0.001$) concentrations and plasma Factor XII activity ($r = -0.48$; $p < 0.001$). Even after correcting for possible bias due to differences in age, sex and PAD, the associations remained significant (Table 3). As expected, aneurysm size shows relatively good prognostic value in predicting aneurysm progression (AUC, 0.83; 95% CI, 0.71–0.96; $p < 0.001$). The prognostic value of the individual markers was not significant, but aneurysm diameter combined with either Factor XII activity or α1-AT, by regression, had minimal effect on the prognostic value in predicting aneurysm progression (AUC, 0.85; 95% CI, 0.73–0.97; $p < 0.001$ and AUC, 0.85; 95% CI, 0.72–0.98; $p < 0.001$, respectively; Fig. 3). Although Ig spot abundance differs significantly between stable and progressive AAA, making this a putative marker for aneurysm progression, serum IgG concentration combined with D_{max} did not improve the ability to predict aneurysm progression (AUC, 0.83; 95% CI, 0.71–0.96; $p < 0.001$).

Discussion

We detected differentially expressed proteins in the serum from patients with stable, progressive and large AAA. These differential proteins are involved in pathophysiological key processes of AAA, such as inflammation (e.g., IgG), extracellular matrix (ECM) remodelling (e.g., α1-AT) or coagulation and fibrinolysis (e.g., Factor XII).

In a recent review, members of the 'Fighting Aneurysmal Disease' Consortium highlighted the role of ILT in AAA pathogenesis.[22] ILT is biologically active with at the luminal interface active fibrinolysis and coagulation; markers of these processes correlate with AAA size.[18,19,23,24] D-dimer is likely to be released as a breakdown product of continuous thrombus remodelling.[25] Furthermore, D-dimer is known to stimulate synthesis and release of pro-inflammatory cytokines from neutrophils and monocytes *in vitro*.[26] Plasma D-dimer concentrations show strong association with AAA presence, diameter and progression.[18–21] This association between D-dimer concentrations and AAA diameter was confirmed in our patient population. We are the first to correlate Factor XII with AAA size. Factor XII is involved in the intrinsic coagulation cascade and is not required for physiological coagulation *in vivo* as Factor XII deficiency does not result in abnormal bleeding. It is hypothesised that Factor XII plays a role in stabilising the fibrin clot. We found an increased expression of Factor XII, combined with a lower Factor XII activity in large compared with small AAA. The fact that both Factor XII and its activity are highly significantly related to aneurysm size suggests an excess consumption of Factor XII. Further research regarding the role of Factor XII in AAA is required to validate our findings.

Neutrophils predominate within the ILT and are a major source of proteases associated with ECM degradation. In this study, we detected and validated higher serum α1-AT concentrations in patients with small compared with large AAA. Alpha-1-AT is an acute-phase protein and serine protease inhibitor, which inactivates enzymes, such as elastase. Decreased amounts of α1-AT in serum facilitate proteolytic ECM degradation, weakening the vascular wall, resulting in dilatation and making it more prone to rupture. In agreement with our study, Schachner et al. demonstrated that α1-AT concentrations are reduced in the

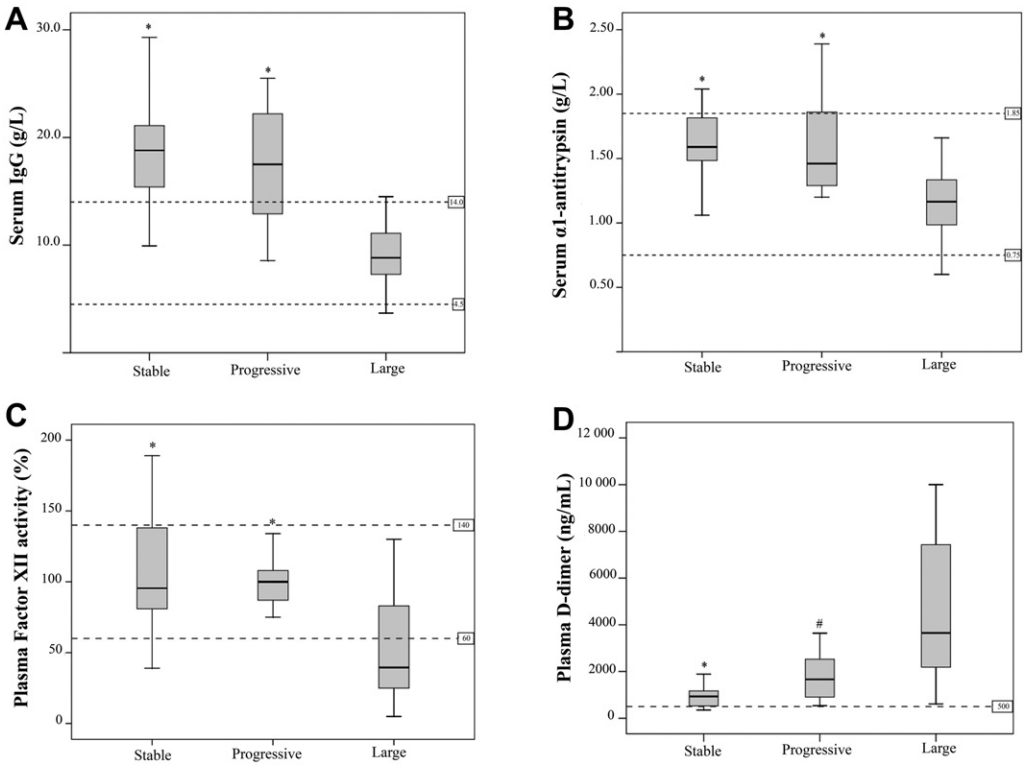

Figure 2 Validation of differential (A) serum IgG (B) serum α-1 antitrypsin (C) plasma Factor XII activity, and (D) plasma D-dimer expression in eighty patients with abdominal aortic aneurysm. Dashed lines show the reference values, as established at our hospital. Significant difference compared to large AAA is indicated by $*p < 0.001$ or $\#p = 0.002$.

vascular wall of ascending aortic dissections compared with healthy aortas.[27] Furthermore, Lindholt et al. and Vega de Céniga et al. both showed a positive relation between AAA progression and serum α1-AT concentrations, but were not able to demonstrate any relation with AAA diameter.[28,29] In contrast to α1-AT, IgG measurements in addition to aneurysm size did not improve the ability to predict aneurysm progression. Correlation between serum α1-AT and IgG concentrations suggests that α1-AT up-regulation in small AAA is associated with inflammation and to a lesser extent with ECM degradation. Inflammation is a key process in AAA formation, as demonstrated by extensive inflammatory cell infiltration,[30] but it remains unclear whether this inflammation is causal or simply a reaction to ECM degradation. Here, we showed the increased expression of serum IgG in small AAA. Based on positive associations between AAA size and cytokines as well as other inflammatory markers,[29,31–33] higher serum IgG concentrations would be expected in large AAA. This discrepancy could be explained by the hypothesis that inflammation has diminished in large AAA and progression is more likely due to haemodynamic factors. This hypothesis is partly supported by a recent publication of Parry and colleagues, in which they observed elevated C-reactive protein (CRP) and fibrinogen levels in men with small AAA reflecting a pro-inflammatory state.[21]

Table 3 Linear regression assessing the individual relation between biomarker and D_{max}.

	B (SE)	*p*-value	Goodness of fit
α1-antitrypsin	−21.29 (4.80)	<0.001	0.381
IgG	−1.66 (0.25)	<0.001	0.505
D-dimer	0.004 (0.001)	<0.001	0.482
Factor XII activity	−0.17 (0.04)	<0.001	0.336

Corrected for possible bias due to age, sex and PAD.

However, some limitations should also be addressed. This proteomic technique has a low resolution for alkaline, less abundant and high-molecular-weight proteins, and a number of differential proteins could not be identified with the presently available techniques. The tremendous dynamic range of protein concentrations in serum limits biomarker discovery using 2D-DIGE and depleting the most abundant proteins has received considerable attention. However, the removal process must be close to 100% efficiency to be interesting and less abundant proteins may also be eliminated during the depletion process, due to carrier function of albumin or a specific removal, resulting in loss of reliable information and potential discrepancies between samples. Furthermore, investigating the correlation of biomarker concentrations, especially Factor XII, and ILT volume would be valuable. Unfortunately, data on ILT volume are only available for a limited amount of patients with small AAA, as aneurysm size was assessed using ultrasound in the majority of patients.

In summary, we report a proteomic study on serum samples of AAA patients. The differentially expressed

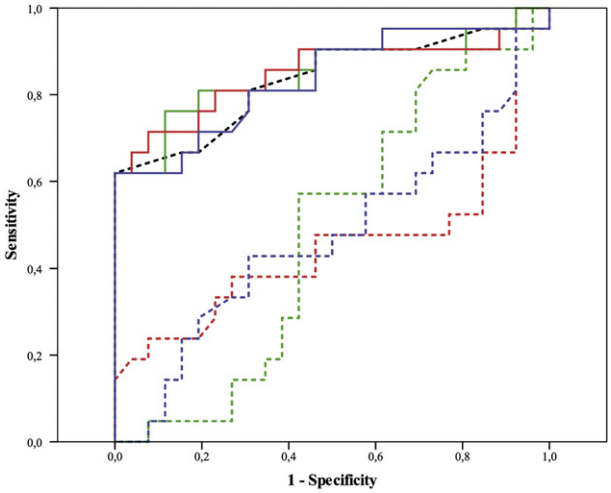

Figure 3 Prediction of aneurysm progression by the individual parameters (dotted line) and parameters combined with aneurysm size (full line). IgG (blue), α-1 antitrypsin (red), Factor XII (green) and aneurysm size (black).

proteins (including Factor XII and α1-AT) are involved in several key features of AAA pathophysiology. Incorporating either Factor XII activity or α1-AT measurements together with aneurysm diameter did not have a marked effect on the prediction of aneurysm progression. Further research, with larger sample sizes, is necessary to investigate whether these or other serum markers of AAA reflect causative factors for aneurysm formation and progression, or are merely secondary effects of aneurysm presence.

Conflict of Interest

None declared.

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