Medial degeneration does not involve uniformly the whole ascending aorta: morphological, biochemical and clinical correlations

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Abstract

Objective: To investigate whether and how the severity of medial degeneration (MD) lesions varies along the circumference of the dilated intrapericardial aorta. Methods: Two groups of aortic wall specimens, respectively harvested 1 cm distal to the non-coronary (NC) sinus (right postero-lateral wall) and to the right coronary sinus (anterior wall) in 22 patients undergoing surgery for dilatation of the intrapericardial aorta associated with aortic valve disease, were separately sent for pathology, morphometry and ultrastructural examination. MD lesions found at histology were classified into three degrees of severity. MD mean degree and morphometric findings in postero-lateral (‘NC’) and anterior (‘coronary’) specimens were compared by paired t-test. Correlation between degree of aortic dilatation at echocardiography and severity of MD was assessed separately for each of the two groups of specimens. After the preliminary results of the morphological study, we decided to send the specimens for biochemical investigation of protein electrophoretic patterns. This was performed in the last seven patients of this series. Results: At histology, MD was found in all cases. A higher mean MD degree was found in the NC group (2.59 ± 0.50 versus 1.59 ± 0.67 in the coronary group; \( P < 0.001 \)). At morphometry, normal smooth muscle cells in the NC specimens were significantly reduced (\( P = 0.012 \)) and the length (\( P = 0.011 \)) and number (\( P = 0.015 \)) of elastic fibres reduced and increased, respectively. Correlation between aortic ratio and MD degree was significant in the NC specimens (\( P < 0.001 \)), not in the coronary ones (\( P = 0.227 \)). Quantitative differences between coronary and NC proteins from the same patient and between coronary proteins from different patients were found at electrophoresis. However, at this stage of the study, the sample was too small to allow for the identification of proteins involved in those differences. Conclusions: MD lesions in dilated intrapericardial aorta are more severe in the right postero-lateral wall area, likely due to haemodynamic stress asymmetry. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Medial degeneration; Aortic valve disease; Aortic dilatation; Bi-dimensional electrophoresis

1. Introduction

Improved imaging techniques provide an early and accurate diagnosis of ascending aorta dilatation associated with aortic valve disease and contributes to increase the observed incidence of this clinical condition. The most common morphological substrate in these cases is medial degeneration (MD) [1–3].

The pathogenesis of aortic wall changes underlying aneurysmal dilatation is still debated: in particular, it has not been established yet whether medial lesions are due to primary connective tissue defects or develop secondary to haemodynamic forces or both [1,4].

Degenerative changes in the aortic elastic media, termed cystic medial necrosis or MD consist of elastic fibre fragmentation, smooth muscle cell loss, pooling of mucoid material within areas depleted of cells and elastic fibres and fibrosis [5]. These findings could either result from damage and repair events occurring in the normal ageing aorta [4,5], or be seen earlier in patients with abnormal post-valvular haemodynamics (excessive injury) and in patients with connective tissue disorders (impaired repair) [6].

New insights into aortic root anatomy and haemodynamics have recently shown a functional and structural asymmetry, which was thought to be strictly related to the biomechanics [7,8]. In surgical series, a regional right
lateral dilatation corresponded to extremely variable degrees of MD changes at histology in both different specimens from the same ascending aorta and within a single specimen [9].

While our previous studies already correlated the degree of aortic dilatation and the severity of histological changes in the aortic valve [10,11], the geometrical distribution of MD within the intrapericardial aorta has not been investigated.

Furthermore, no biochemical study on the changes in protein patterns underlying morphological findings has been issued to date.

The aim of our study was to investigate the pattern of expression of MD in the aortic wall of patients with non-complicated dilatation of the intrapericardial aorta associated to aortic valve disease, and to analyse the correlations between echocardiographic, surgical and morphological findings. Moreover, after the preliminary results of the morphological study, a protocol for biochemical analysis of the protein components of the intrapericardial aortic wall was developed. This new investigative approach to the degenerative disease of the aortic media has been proposed here and the preliminary results of our biochemical study have been reported.

2. Materials and methods

2.1. Patients

Between March 1999 and October 2000, aortic wall specimens were collected from 22 consecutive patients undergoing surgery for aortic valve disease (regurgitation in four patients, stenosis in 15, both in three) with associated ascending aortic dilatation. Patients with atherosclerotic aneurysm, aortitis, infective endocarditis or diagnosed primary connective tissue disorders (such as Marfan syndrome) were excluded from the study. The series included 20 males and two females (M:F ratio 10:1) with a mean age of 56.5 ± 9.4 years (range: 42–75). Body surface areas of patients were calculated according to the Dubois formula (Table 1). In eight patients the whole ascending aorta was replaced, while in the other 14 ascending aortoplasty was performed. The aortic valve was excised and replaced with a mechanical bileaflet valve in all cases: in 18 patients it was found to be structurally diseased, while in four cases with no gross leaflet lesions a severe dilatation of the aortic root with severe valve incompetence was present and no valve-sparing procedures were attempted.

<table>
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<tr>
<th>Patient</th>
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<td></td>
<td>56.5 ± 9.4</td>
<td>1.68 ± 0.15</td>
<td>2.59 ± 0.50</td>
</tr>
</tbody>
</table>

2.2. Echocardiographic diagnosis

Echocardiographic measurements were performed using standard views, and by only two experienced examiners. Transvalvular flow velocities were measured using continuous-wave Doppler. Pressure gradients were then performed after correction for left ventricular outflow tract velocities using the complete Bernoulli equation. Measurements were taken at haemodynamically stable conditions; results were averaged from three subsequent measurements in sinus rhythm. Aortic valve incompetence was graded as mild if the regurgitant jet did not exceed 1 × 2 cm, moderate if it did not exceed the middle of the left ventricle and/or the tips of the papillary muscles and severe if it exceeded the middle of the left ventricle.

Ascending aortic diameters were measured at the level of the aortic sinuses, sino-tubular junction and ascending aorta at its widest diameter from the parasternal window at end-diastole. In order to obtain comparable echocardiographic data, predicted aortic root dimensions were calculated by the regression formula described by Roman et al. [12] and an aortic ratio was computed as measured diameter (maximum dilatation among sinusual level and sino-tubular junction) divided by predicted diameter. Ascending aortic surgery was indicated in case of aortic ratio reaching 1.5 or more. Patient demographics and aortic ratios are shown in Table 1.

2.3. Morphological study

At surgery, samples were harvested about 1 cm distal to the sino-tubular junction level in the two areas corresponding to the right and the non-coronary (NC) sinuses of Valsalva, right postero-lateral wall (specimens named ‘NC’) and anterior wall (named ‘right coronary’ (RC)),
respectively. Also the explanted aortic valve leaflets were sent for histology in all cases.

All aortic wall specimens and valve cusp specimens were obtained by surgical excision perpendicular to the annulus. Specimens from each aorta were separately stored in batches labelled with a progressive number and sent for histological examination. The pathologist was blinded to the register relating each specimen number to its original site within the aortic circumference.

Specimens were embedded in paraffin, into 4-μm thick sections, and stained with haematoxylin–eosin, periodic acid-Schiff (PAS), Weigert–Van Gieson’s stain and orcein for elastic fibres, Alcian–PAS, Alcian–Weigert stain for elastic fibres, von Kossa’s stain. Gram’s stain was also performed to exclude infective endocarditis. In the last eight cases immunohistochemical methods (PAP-staining according to Sternberger) were also employed to find apoptotic cells expressing CD95 surface antigen.

For each specimen, histological changes of MD were evaluated according to the criteria of Schlatmann and Becker [5,6]: cystic MD, defined as pooling of mucoid material; elastic fragmentation characterised by destruction of elastin lamellae; fibrosis, defined as replacement of smooth muscle cells with collagen proliferation; medionecrosis, defined as areas with apparent nuclei loss. The above-mentioned changes were ranked in three different grades: grade 1 (mild), grade 2 (moderate) and grade 3 (severe).

Morphometric analysis was performed by using two programs (RM 2100 or RM 5200) of a computer assisted image analysis system (VIDAS Kontron Elektronik ZEISS). For each specimen, ten microscopic fields (620×) were randomly selected, the number of elastic fibres, of total smooth muscle cells (normal and degenerated together) and of normal smooth muscle cells alone was counted and mean values were computed. The length of elastic fibres, considering their angulation, and their density per field were also measured.

The ultrastructural study was performed by using 2-mm thick fragments, fixed in paraformaldehyde, post-fixed in osmium tetroxide, embedded in Epon 812-resin, cut into sections (600 Å in thickness) and stained with lead citrate and uranyl acetate. The transmission electron microscopy (ZEISS EM 902) was used. Ultrastructural analysis was performed using the same programs and image analysis system as above. For each case, five fields (620×) were selected, the elastic fibres and the normal (non-degenerated) smooth muscle cells were counted and mean values were computed. The length of elastic fibres, considering their angulation, and their density per field were also measured.

2.4. Statistical analysis

In order to evaluate differences in MD severity between the two aortic sites, degrees of MD were coded as discrete variables assigned to each specimen (two groups of values composed by the same patients) and comparisons between RC and NC specimens were made by means of paired t-test. Paired t-test was also employed to compare morphometric findings between the two groups of specimens.

Moreover, patients were divided into three groups according to the aortic ratio (>1.7, 1.6–1.7 or <1.6). For each of the two groups of specimens the correlation between class of aortic ratio and degree of MD was assessed by means of chi-square test with Yates’ continuity correction.

2.5. Biochemical study

In the latter part of our study we began sending specimens also for biochemical analysis. Using bi-dimensional polyacrylamide gel electrophoresis (2D-PAGE), we attempted to separate the complex protein mixture of the ascending aortic wall and to identify possible quantitative and/or qualitative protein differences between the two sample sites.

Specimens (10–20 mg), harvested as reported above, were frozen in liquid nitrogen and stored at −80°C. Then they were homogenised in 50 mM Tris–HCl buffer (0.5 ml × mg of sample), pH 7.4, with 150 mM NaCl, containing 10 mM EDTA, 1 mM PMSF, 0.3 mM TPCK and 10 μg/ml aprotinine as proteinase inhibitors. The homogenate was centrifuged at 4200× g and 4°C for 30 min in a F1010 rotor (Beckman centrifuge GS-15R). Protein concentration of supernatants from each sample was then determined using the BCA assay reagent (Pierce, Rockford, IL, USA), in order to load the gel with equal amounts of protein. To this end, 0.6 mg of protein supernatant were precipitated with 20% TCA (final concentration), dissolved in denaturing solution of 8 M urea, 2% CHAPS, 2.5% BFB, 2% IPG buffer and 2.8 mg DTT/ml (added just prior to use) and subjected to 2D electrophoresis as described by Bjellqvist et al. [13].

The first dimension (isoelectric focusing) used Immobiline Dry Strip pH 3–10 L (Amersham Pharmacia Biotech) and the second dimension (SDS-PAGE) used 15% gels, which were stained with silver nitrate. When adequate, protein spots were electroblotted onto PVDF membrane and subjected to Edman degradation on a Procise Model 91 sequencer (Applied Biosystem) to obtain their N-terminal sequence for identification.

3. Results

At echocardiography, aortic valve regurgitation was found in four patients (moderate in one, severe in three), aortic stenosis in 15 (moderate in four, severe in 11) and mixed disease in three.

At morphological studies, the right postero-lateral aortic wall (distal to the NC sinus) was in all cases enlarged and thinned (Fig. 1); at transillumination, the wall was transparent in all cases. The anterior wall (distal to the coronary sinus) showed normal thickness in 19 patients, while it was slightly thinned in the other three.
At histology, MD was found in all cases (Figs. 2 and 3). A more severe involvement of the aortic portion corresponding to the NC sinus was observed in 18 patients (grade 3 versus grade 1 or 2 in 11, grade 2 versus grade 1 in seven) and a similar degree of involvement of both sites in four (Table 1). The mean degree of MD in NC specimens was $2.59 \pm 0.50$ versus $1.59 \pm 0.67$ in the coronary specimens ($P, 0.001$).

Pathology examination of valve leaflets showed structural aortic valve disease in 18 cases: dystrophic calcific valve disease in ten patients, chronic rheumatic valve disease in seven and floppy aortic valve in one. In four patients, the valve showed no changes although at surgery it had proved to be severely incompetent due to great annular dilatation.

The results of the morphometric analysis are summarised in Table 2. Smooth muscle cell loss was found in 12 out of 22 patients. Elastic fibre length was reduced in 16 out of 22 patients while their number per field was increased in the same cases. This was due to elastic fibre fragmentation. Differences between NC and coronary specimens as to the number of normal (non-degenerated) smooth muscle cells ($P = 0.012$) and the length ($P = 0.011$) and number ($P = 0.015$) of elastic fibres were found to be statistically significant. Disruption and fragmentation were more severe and the elastic fibres were shorter and more numerous in NC sinus than in coronary sinus in 16 patients. Morphological findings were consistent with morphometric data in eight patients.

No case of CD 95 expression (apoptosis) was observed in either coronary or NC sinuses of the eight patients in whom immunohistochemistry was performed.

The ultrastructural examination, performed on eight cases, confirmed the elastic fibre damage and smooth cell necrosis with a structural alteration and formation of pseudocystic pooling of mucoid material; besides, thin fibrils and skins of fibrillin were observed. The smooth muscle cells near the pseudocysts showed degenerative changes such as hydropic changes (dilated endoplasmic reticulum, mitochondrial swelling and crystolysis) and focal dissolution of actin and myosin, sometimes with myelin-like figures. Such ultrastructural changes were strongly marked in NC sinus in all cases and were consistent with morphological ones in six cases.

The correlation between the aortic ratio range and the grades of MD lesions was found to be statistically significant only in the group of specimens corresponding to the right postero-lateral aortic wall ($P < 0.001$), while the
degree of aortic enlargement did not significantly correlate with the degree of involvement of the anterior wall ($P = 0.227$) (Table 3).

The biochemical study was performed in the last seven patients of the study (16–22 in Table 1). Fig. 4 reports an example of 2D separation which allowed for comparison of the protein patterns between coronary and NC specimens: quantitative differences in several protein spots were revealed. Most of these differences consisted in larger spots in the coronary specimen versus the corresponding spots in the NC one. In some instances, comparing coronary specimens from different patients, not only were quantitative differences in some corresponding spots found, but also the presence of different spots was observed (Fig. 5).

Blotting experiments aiming to identify the protein spots did not succeed, due to the poor amount of proteins contained in the samples. However, large spots were identified (serum albumin, actin, haemoglobin alpha and beta chain; Fig. 5), suggesting the validity of 2D electrophoresis for this kind of analysis, provided a higher protein amount is used.

### 4. Discussion

Our results describe a definite pattern of distribution of MD severity within the aortic wall. This pattern is consistent both with the recent finding of asymmetry in the functional anatomy of the aortic root [7,8,14] and with the proposed stress origin of MD lesions [4–6].

Necropsy series by Shennan and Hirst [15–17] already showed that dissection of the ascending aorta usually does not involve the entire circumference equally. A longitudinal segment facing the main pulmonary artery tends to remain unaffected while the right lateral, anterior and posterior portions of the vessel are dissected. Studies on aortic root models or on autopsy samples [8,14], showed that the structures of the NC sinus are the largest, followed by those of the right and then those of the left. Grande et al. [7], studying aortic root biomechanics, found that valve stresses are higher on the NC leaflet.

Our results not only confirmed the relationship between degree of aortic dilatation and aortic wall changes previously observed both by us [10,11] and other authors [18], but also added an interesting information that such relationship was found to be more significant for the right postero-lateral aortic wall than for the anterior one, although the statistical significance of this finding needs to be validated in larger series. However, the more severe expression of MD changes in the postero-lateral aortic wall could be correlated to the increased risk of aortic wall dissection or rupture at that site.

The pathogenesis of MD changes is still debated. Gore and Hirst [19] and Edwards [20] considered the fragmentation of the elastin framework of the aortic media as an expression of connective tissue defects in patients under 40 years of age with dissecting aneurysm. On the other

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**Table 2**

<table>
<thead>
<tr>
<th>MD changes</th>
<th>Mean RC</th>
<th>Mean NC</th>
<th>$P$</th>
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<td>No. of smooth muscle cells</td>
<td>526.0 ± 184.1</td>
<td>431.5 ± 197.2</td>
<td>0.246</td>
</tr>
<tr>
<td>No. of normal smooth muscle</td>
<td>473.6 ± 188.6</td>
<td>274.5 ± 162.0</td>
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</tr>
<tr>
<td>No. of elastic fibres</td>
<td>200.9 ± 91.3</td>
<td>366.4 ± 210.4</td>
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<tr>
<td>Minimum length of elastic fibres</td>
<td>0.97 ± 0.43</td>
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<td>0.001</td>
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<tr>
<td>Greatest length of elastic fibres</td>
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<td>11.3 ± 3.29</td>
<td>0.168</td>
</tr>
<tr>
<td>Mean length of elastic fibres</td>
<td>4.47 ± 2.1</td>
<td>2.69 ± 1.12</td>
<td>0.011</td>
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</table>

*RC, right coronary specimens; NC, non-coronary specimens.*
hand, in normal aortas, elastin fragmentation is present in almost all cases and its severity increases with age [5,6]. Areas with fragmented elastin fibres also exhibit a concomitant increase in mucopolysaccharides and reparative changes such as a proliferation of smooth muscle cells and the formation of connective tissue fibres [5,6]. Therefore, Schlatmann suggested that MD changes may be the histopathological expression of a traumatised media.

The two pathogenetic hypotheses could correspond to two morphological types of ascending aorta dilatation: the first consists of a concentric or ‘pear’ dilatation of aortic root and annulus caused by a defect of elastin tissue due to a congenital connective pathology. Aortic valve degenerative disease such as floppy valve is sometimes associated [10]. The second type is an eccentric aortic root dilatation more strongly expressed in the postero-lateral aspect of the vessel, caused by haemodynamic stresses due to aortic valve dysfunction. Aortic valve diseases such as rheumatic disease, dystrophic calcifications and bicuspid valves are possible associated pathologies.

No statistical analysis of correlation between the severity of valve disease and the degree of MD could be performed in this study due to the small population enrolled so far; in our opinion, it would have been incorrect to consider in the same group those patients with aortic regurgitation and those with aortic stenosis or mixed disease. A larger population will allow for patient assignment to two or more groups depending on the type of valve disease and for further analysis on correlations between post-valvular rheology changes and aortic wall lesions.

Since histopathology changes are similar in the two types of aortic dilation, molecular biology and genetic studies are needed in order to try to differentiate primitive from secondary medial changes. To this purpose, the identification of apoptosis could have added some information. The absence of CD95 positivity in our cases may be due to a long fixation time (immunostain was performed only in eight retrospective specimens); therefore this method of investigation should be extended to fresh or frozen fixed specimens.

It seemed to us that a biochemical approach could provide interesting insight into the lesions underlying MD changes, and therefore we decided to apply biochemical methods of investigation in that subset of aortic dilation patients who show asymmetrical involvement of the aortic wall at histol-

Table 3
Histological findings grouped according to the aortic ratio (column percentages and P values are reported)*

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<th></th>
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<td>2 (22.2%)</td>
<td>5 (38.5%)</td>
<td>4 (36.4%)</td>
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<td>8</td>
<td>0</td>
<td>9 (61.5%)</td>
<td>13</td>
<td>2 (18.2%)</td>
<td>4 (44.5%)</td>
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<tr>
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<td>13</td>
<td>11</td>
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<tr>
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<td>&lt;0.001</td>
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* AR, aortic ratio ranges; N, number of patients; RC, right coronary specimens; NC, non-coronary specimens.

Fig. 4. Bi-dimensional separation of proteins from the coronary sinus specimen (left panel) and NC sinus specimen (right panel) of one patient. Arrows indicate quantitative differences among spots; attempts to identify those proteins were unsuccessful (see text).
ogy and morphometry, that is patients with associated aortic valve disease.

Our biochemical studies were carried out in seven patients. Because of the small number of patients, no statistically significant conclusion can be drawn from the preliminary results of the biochemical study yet. Nevertheless some evidence arose, which may represent a validation to this methodology for further studies. The protein patterns of NC and coronary specimens showed quantitative differences in some protein spots. Another interesting finding was that the total protein spot number is higher in samples with grade 1 MD than in those with grade 3 MD.

Many authors have reported that structural changes in insoluble proteins such as collagens [21] and elastin [22] play a role in human vessel dilatation. Other proteins, in particular the soluble ones, were the object of our study. Such proteins may be enzymes which, if over-expressed or lacking, could bring about changes in the structural proteins of the aortic wall [23]. However, by using a higher amount of starting material for 2D separation, other proteins, such as collagen [24] and fibrillin [25] could be investigated at immunoblotting.

Our histological results are consistent with previous anatomo-functional observations, showing an asymmetrical involvement of the proximal aorta in MD lesions. Identifying the structures of the involved proteins could be an enlightening advancement in the debate on the pathogenesis of the dilatation of the intrapericardial aorta and could also better explain how the asymmetry in biomechanics may be related to the asymmetry in histological changes.

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References


