Histopathological Grading of Ascending Aortic Aneurysm: Comparison of Patients with Bicuspid versus Tricuspid Aortic Valve

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Background and aims of the study: Bicuspid aortic valve (BAV) is a common inherited condition that is often accompanied by ascending aortic aneurysm. A high level of histological wall abnormalities was reported to be present in non-dilated aortas of patients with BAV. In patients with tricuspid aortic valve, there appears to exist a direct relationship between the diameter of the ascending aorta and degree of histopathological aortic wall abnormalities. Whether this situation exists in patients with BAV has not yet been investigated.

Methods: Surgical and medical records of all patients undergoing surgery of the ascending aorta were reviewed. A total of 65 patients was identified in whom an aortic wall specimen was obtained intraoperatively. These specimens were systematically re-evaluated, and graded according to the severity of seven histopathological conditions: fibrosis, atherosclerosis, medionecrosis, cystic medial necrosis, smooth muscle cell orientation, elastic fiber fragmentation, and inflammation.

Results: BAVs were present in 26 patients (40%). Patients with BAV had significantly less aortic wall alterations than patients with tricuspid aortic valves (p <0.001) in all variables examined. The severity of aortic wall abnormalities was significantly dependent on aortic diameter in patients with BAV as well as tricuspid aortic valve (p = 0.036 and 0.019), but dependent on age (p = 0.009) only in patients with tricuspid aortic valve.

Conclusion: The study results provide evidence that ascending aortic aneurysm in patients with BAV differs clinically and histologically from that in patients with tricuspid aortic valve. Further studies are needed to elucidate the impact of inherited and acquired aortic wall abnormalities on the development of aneurysms.

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the ascending aorta was performed at the authors’ department. Among patients, one aortic leaflet was present in one patient (0.6%), while two leaflets were present in 42 patients (25.1%) and three leaflets in 96 (57.5%). An unknown number was present in 28 patients (including 10 who had undergone prior aortic valve replacement). Patients with bicuspid aortic valve were significantly younger than those with tricuspid aortic valve (53 ± 14 versus 62 ± 13 years, respectively; p < 0.001). Among patients with either a bicuspid or tricuspid aortic valve, an aortic wall specimen was excised from the anterior aspect of the convexity of the ascending aorta at the time of surgery in 65 cases, and these specimens form the basis of this report.

**Table I: Clinical characteristics of all patients.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Aortic valve</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bicuspid</td>
<td>Tricuspid</td>
</tr>
<tr>
<td>Male/female (n)</td>
<td>18/8</td>
<td>25/14</td>
</tr>
<tr>
<td>Age (years)’</td>
<td>53 ± 15</td>
<td>57 ± 14</td>
</tr>
<tr>
<td>Hypertension (n)</td>
<td>10 (42)</td>
<td>24 (63)</td>
</tr>
<tr>
<td>Diabetes mellitus (n)</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Marfan syndrome (n)</td>
<td>0</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Aneurysm diameter (mm)’</td>
<td>61 ± 11</td>
<td>58 ± 6</td>
</tr>
<tr>
<td>Dissection (n)</td>
<td>2 (8)</td>
<td>27 (69)</td>
</tr>
<tr>
<td>Need for aortic valve surgery (n)</td>
<td>22 (85)</td>
<td>28 (72)</td>
</tr>
<tr>
<td>Type of aortic surgery (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replacement</td>
<td>39 (100)</td>
<td>25 (96)</td>
</tr>
<tr>
<td>Wrapping</td>
<td>0</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. Values are mean ± SD.

**Histopathological evaluation**

The resected material was fixed in 4.5% pH-buffered formalin for approximately 24 h. Representative portions of the resected material were selected macroscopically for further processing. If necessary, decalcification of atherosclerotic lesions was undertaken by use of Ossa fixona solution (Diagonal, Muenster, Germany). The tissue was processed for light microscopy, embedded in paraffin blocks, and sections (4 µm thickness) were taken from each specimen. Sections were stained with hematoxylin and eosin, elastica-van Gieson, Alcian blue and Masson’s trichrome stains. For the purpose of this study, all specimens were re-evaluated by two experienced histopathologists who...
were blinded to the clinical data. The following histological alterations were analyzed semiquantitatively: (1) fibrosis (defined as an increase in interstitial collagen); (2) atherosclerosis (defined as the presence of intimal fibrous plaques and/or complex or complicated atheromas); (3) medionecrosis (defined as a focal loss of smooth muscle cell nuclei in the media); (4) cystic medial necrosis (defined as mucoid material accumulation); (5) changes in smooth muscle cell orientation; (6) elastic fragmentation (defined as focal fragmentation of elastic lamellae in the media); and (7) periaortic inflammation (defined as the presence of inflammatory cells). Each variable was graded from 0 (no change) to 3 (most severe change) when examined at a magnification of \( \times100 \) or \( \times200 \), using an Olympus microscope (Olympus BX 50, Japan). The grades were determined on the basis of the worst area observed. Examples are shown in Figures 1 and 2. The criteria for histological grading were used as proposed by Schlatman and Becker (13), Klima et al. (14) and de Sa et al. (10) and are detailed in Appendix I. The sum of the results of all variables was calculated for each individual patient, and this was referred to as the aortic wall score.

### Statistical analysis

Data were presented as absolute numbers and relative percentages or mean (± SD), except where otherwise stated. Relative frequencies were compared using Fisher’s exact test; continuous data were compared using the Mann-Whitney \( U \)-test. Linear regression analysis was performed with the aortic wall score as dependent variable, and age and aortic diameter as independent variables. All analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA).

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### Table II: Results of histopathological evaluation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Grade</th>
<th>Bicuspid (n)</th>
<th>Tricuspid (n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis</td>
<td>None</td>
<td>19 (73)</td>
<td>11 (28)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>3 (12)</td>
<td>15 (39)</td>
<td>0.002</td>
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<td></td>
<td>II</td>
<td>3 (12)</td>
<td>11 (28)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>1 (4)</td>
<td>2 (5)</td>
<td></td>
</tr>
<tr>
<td>Atherosclerosis*</td>
<td>None</td>
<td>12 (46)</td>
<td>11 (29)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>10 (39)</td>
<td>9 (24)</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2 (8)</td>
<td>5 (13)</td>
<td></td>
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<tr>
<td></td>
<td>III</td>
<td>2 (8)</td>
<td>13 (34)</td>
<td></td>
</tr>
<tr>
<td>Medionecrosis</td>
<td>None</td>
<td>18 (69)</td>
<td>9 (23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>5 (19)</td>
<td>10 (26)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1 (4)</td>
<td>11 (28)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>2 (8)</td>
<td>9 (23)</td>
<td></td>
</tr>
<tr>
<td>Cystic medial necrosis</td>
<td>None</td>
<td>16 (62)</td>
<td>10 (26)</td>
<td>0.008</td>
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<td></td>
<td>I</td>
<td>5 (19)</td>
<td>13 (33)</td>
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<td>II</td>
<td>3 (12)</td>
<td>10 (26)</td>
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<td></td>
<td>III</td>
<td>2 (8)</td>
<td>6 (15)</td>
<td></td>
</tr>
<tr>
<td>SMC orientation+</td>
<td>Normal</td>
<td>24 (92)</td>
<td>22 (63)</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>1 (4)</td>
<td>7 (20)</td>
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<td>1 (4)</td>
<td>6 (17)</td>
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<td>III</td>
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</tr>
<tr>
<td>Elastic fragmentation†</td>
<td>None</td>
<td>12 (46)</td>
<td>5 (13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>6 (23)</td>
<td>6 (16)</td>
<td></td>
</tr>
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<td>6 (23)</td>
<td>6 (16)</td>
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<td></td>
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<td>2 (8)</td>
<td>21 (55)</td>
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<tr>
<td>Inflammation</td>
<td>None</td>
<td>19 (73)</td>
<td>11 (28)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>5 (19)</td>
<td>15 (39)</td>
<td></td>
</tr>
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<td></td>
<td>II</td>
<td>1 (4)</td>
<td>6 (15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>1 (4)</td>
<td>7 (18)</td>
<td></td>
</tr>
<tr>
<td>Aortic wall score‡</td>
<td></td>
<td>3.8 ± 3.8</td>
<td>9.2 ± 4.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

*Variable not evaluated sufficiently in one patient with tricuspid aortic valve.

†Variable not evaluated sufficiently in four patients with tricuspid aortic valve.

‡Values are mean ± SD.

SMC: Smooth muscle cell.
Results

The demographic and surgical data of patients are listed in Table I. A total of 26 patients (40%) had bicuspid aortic valve.

Five patients had no detectable histopathological changes; four of these had bicuspid aortic valve (p = 0.15). The mean aortic wall score was 7.1 ± 5.1. In patients with tricuspid aortic valve, regression analysis revealed a significant association between age at operation, ascending aortic diameter, and aortic wall score (aortic wall score = 0.42 × age + 0.33 × diameter; p = 0.009 and 0.036, respectively). In contrast, patients with a bicuspid aortic valve showed a significant association between aortic diameter (but not age) and aortic wall score (aortic wall score = 0.49 × diameter; p = 0.019). The association between aortic wall score and aortic diameter is shown in Figure 3.

Among patients with hypertension or diabetes mellitus, all of the examined histopathological variables were of similar severity. Patients with aortic dissection had a more severe extent of cystic medionecrosis (p = 0.017) as compared with patients with aneurysm, but all other variables examined were of similar severity. The results of the histopathological evaluation for patients with bicuspid versus tricuspid aortic valve are listed in Table II. Patients with bicuspid aortic valve had a significantly lower mean aortic wall score (3.8 ± 3.8 versus 9.2 ± 4.6; p <0.001) due to significantly less severe histopathological changes in all variables examined.

Discussion

The results of this retrospective study suggest that at the time of aortic surgery - patients with a bicuspid aortic valve have less severe aortic wall abnormalities according to histological standard criteria than those patients with a tricuspid aortic valve, despite the presence of similar degrees of aortic dilatation. In addition, these results confirm that bicuspid aortic valve is frequent among patients undergoing surgery for diseases of the ascending aorta, and that a wide variety of aortic wall abnormalities of the ascending aorta can be observed in patients with tricuspid as well as bicuspid aortic valve.

In patients with tricuspid aortic valve, there appears to be a direct positive correlation between the degree of ascending aortic dilatation and the degree of histological aortic wall abnormalities (11,12), but whether the same situation exists among patients with bicuspid aortic valve has not yet been investigated. In the present study, less severe histopathological changes were found in patients with bicuspid aortic valve despite a similar degree of aortic dilatation; thus, these results may be interpreted in such a way that the ‘true’ lesion in the aorta of patients with bicuspid aortic valve is not identified using standard light microscopy criteria and that the mechanism of dilatation may differ from that in patients with tricuspid aortic valve. In accordance with the first hypothesis, Parai et al. (15) have shown there to be subtle (but significant) differences regarding the amount of elastic tissue between the aorta of patients with bicuspid and tricuspid aortic valve which could only be identified using morphometry. Recently, Bauer et al. (16) confirmed that patients with bicuspid aortic valve have less elastic tissue in their ascending aorta, while Nistri et al. (17) found evidence that the aorta of patients with bicuspid aortic valve appears to be stiffer when compared with that in patients with tricuspid aortic valve. Bonderman et al. (18) found evidence of a generally increased rate of apoptosis in patients with bicuspid aortic valve, whereas in patients with tricuspid aortic valve the rate of apoptosis was elevated only in dilated aortas.

In studying patients with non-dilated aortas, de Sa et al. (10) reported that patients with bicuspid aortic valve had more severe aortic wall abnormalities than patients with tricuspid aortic valve. These authors also found that patients with bicuspid aortic valve frequently have severe wall abnormalities in the main pulmonary artery, which develops from the same embryological structures as the ascending aorta (19). This apparent contrast to the results of the present study cannot be explained easily. Indeed, some evidence was found of a direct positive correlation between the degree of aortic wall abnormalities and aortic diameter in patients with bicuspid as well as tricuspid aortic valve, but this occurred on a generally lower level in patients with bicuspid aortic valve. As patients with non-dilated aortas were excluded from study...
the present study, the possibility of a two-phase model cannot be excluded: a constant first phase (a constant degree of aortic wall abnormalities in the range of normal aortic diameters) followed by a steady increase of aortic wall abnormalities after a certain degree of dilatation has been exceeded. Further studies should be conducted in order to determine the relationship between aortic diameter and associated aortic wall abnormalities.

Most patients with bicuspid aortic valve will never experience aortic dilatation, dissection or rupture, though some will require ascending aorta replacement early in life (2). Besides genetic differences among patients with bicuspid aortic valve, other factors such as postvalvular flow may also contribute to this highly variable prognosis.

More recent studies have indicated that the aortic root is an asymmetric, highly complex structure (20,21). Flow in the ascending aorta is usually eccentric, and studies on prosthetic aortic valves have shown that orientation of the leaflets has a major impact on blood velocity and the presence of turbulence in the ascending aorta (22,23). The precise orientation and morphology of the bicuspid aortic valve varies widely (24-26), and thus the morphology of a bicuspid valve may cause abnormal blood flow in the ascending aorta, even in the absence of a significant degree of valvular disease. Whether these proposed flow disturbances are a cofactor in the development of ascending aortic dilatation/dissection has not yet been investigated, but experimental studies have provided some evidence that flow disturbances or hemodynamic stress can cause dilatation of vessels (27,28). The impact of flow disturbances on histological aortic wall alterations is, at present, unknown.

Study limitations
The main limitation of the present study was its retrospective design. Some selection bias was clear: a histopathological examination was more likely to be ordered in younger patients, elective settings, or when no etiology of a dissection (such as bicuspid aortic valve or prior valve replacement) was apparent. This bias might explain the low incidence of aortic dissection in patients with bicuspid aortic valve: 18 of the 28 patients with an unknown number of aortic valve leaflets had acute type A aortic dissection. However, the results remain virtually unchanged if all patients with aortic dissection are excluded from the analysis. Furthermore, aortic wall abnormalities are not symmetrically distributed among the ascending aortic circumference (29), although the total aortic circumference was not examined. Nonetheless, any sampling error is likely to occur at random and it is likely that the main finding was unaffected by this limitation.

In conclusion, the present study provides some evidence that ascending aortic aneurysm in patients with bicuspid aortic valve differs histologically from that in patients with tricuspid aortic valve. Further studies should be conducted in order to elucidate the impact of inherited and acquired (for example, by age or flow disturbances) aortic wall abnormalities on the development of aneurysms.

References
13. Schlatman TJM, Becker AE. Histologic changes in the normal aging aorta: Implications for dissecting

Meeting discussion

DR. CRISTINA BASSO (Padova, Italy): It seems that the histological samples were not re-evaluated by the pathologist. Which staining do you normally use to investigate aortic wall pathology?

DR. J. F. MATTHIAS BECHTEL (Lübeck, Germany): The specimens were stained with hematoxylin and eosin, and elastica-van Gieson; they were paraffin-buffered and sectioned at 10 µm thickness.

DR. BASSO: When you specified the aortic wall abnormalities you mentioned only mucoid degeneration and cystic necrosis - not the elastic fibers. What was the elastic fiber architecture of the aortic wall?

DR. BECHTEL: These studies were not carried out in all patients, so we only focused on the standard protocol.

DR. BASSO: But this is an issue that we must look for in aortic wall pathology in aortic aneurysm.

DR. BECHTEL: I agree with that, but in our opinion the absence of any evident histologic abnormality in these patients doesn’t exclude the presence of aortic wall abnormalities - but it does suggest that other factors are operative. In my opinion, these factors are synergistic to aortic wall abnormalities.

DR. JAGDISH BUTANY (Toronto, Canada): Thank you for raising such a provocative subject. Do you know how much of the aorta was excised at surgery?

DR. BECHTEL: The aortic wall specimen was usually taken from the anterolateral aspect of the convexity of the aneurysm. Among the patients with bicuspid aortic valve there were no statistical imbalances with regards to valve surgery or the type of ascending aortic aneurysm surgery performed.

DR. BUTANY: There have been many reports showing that as we grow older there is a sequential set of changes in the aortic wall - none of us will escape that. So those changes will be present regardless of any other changes. I trust you are aware of that. Another point is, did you obtain a ring of aortic tissue to examine for histopathology? The exaggerated changes that you showed - the shelf-like change - is more than likely a change as a consequence of aortic incompetence rather than a pri-
mary change in the aortic valve cusp. So changes in the aortic wall are less likely, if not unlikely, to be related to that shelf-like change in the aortic valve cusp. The changes in the aortic wall probably occurred earlier than the development of that shelf in the aortic cusps.

DR. BECHTEL: We can’t exclude that, but as I mentioned we did not have circumferential specimens. They were usually from the anterolateral aspect of the convexity.

DR. BUTANY: In a study that we published in Toronto, we showed that even if you didn’t see significant morphological changes, you find many fibrillin changes - but you have to perform much more extensive studies.

DR. BECHTEL: I am aware of those studies, but this is a retrospective study that is sort of provocative. We were not able to re-evaluate the specimens, or to carry out more extensive studies until now.

DR. KARYN KUNZELMAN (Madison, Wisconsin, USA): In the follow up to that, you have acknowledged that your study is somewhat limited by the lack of histological data due to its retrospective nature. Are you going to continue this in a prospective manner to answer some of these questions that are being raised?

DR. BECHTEL: Yes - these results only included data up to the end of 1999 when the case patient was operated on.

DR. KUNZELMAN: Have you considered that mechanical stress alterations rather than just hemodynamic changes might alter the properties of the root or the ascending aorta?

DR. BECHTEL: I won’t speculate too much on that. I used the term ‘hemodynamics’ because I wasn’t quite sure what term should be used. I am not sure exactly what caused these aneurysms. We speculate that turbulence is developing behind the valve, and this causes the aneurysms to develop, but it is unclear whether this should be called mechanical stress or whether it is hemodynamic inasmuch as you can influence it by decreasing dp/dt.

DR. DANIEL LOISANCE (Creteil, France): Thank you for raising a very difficult issue - I am very impressed by your provocative conclusions. However, they don’t fit in with what we observe clinically, or what we see when we examine the specimen very carefully. Clinically, how can you explain the appearance of aortic dilatation following aortic valve replacement in these patients who had initially a bicuspid valve? It could be the hemodynamic parameters that explain this secondary dilatation. A second observation is that when we examine these aortic tissues they appear extremely abnormal - not only microscopically but also in their subcellular structures. For instance, glycosaminoglycan production is extremely different, TIMP (tissue metalloproteinase) is extremely different, TIMP expression is very different, and MMP-1 (matrix metalloproteinase-1) and MMP-3 are also extremely different. So there is more and more convincing evidence that there is some kind of gene function behind that, and that the hemodynamic parameters may offer a secondary explanation. What is you opinion about that?

DR. BECHTEL: To answer your last point, many excellent reports have been published recently providing evidence that there is a genetically determined aortic wall abnormality in patients with bicuspid aortic valve. A recent paper from De Sa and colleagues in Toronto showed pulmonary wall abnormalities also occur very frequently, but I don’t want to challenge all these findings. Nevertheless, we thought it possible that other factors might be operative in some patients. For example, hemodynamics may have a major impact on the question of whether or not these aortic wall abnormalities will develop into an aneurysm, or simply be present. To return to your first point, there are studies on disk orientation in bileaflet prosthetic heart valves that show that the exact orientation of the valve has a major impact on turbulence behind the valve. It is possible that this could be why some, but not all, patients with bicuspid aortic valve develop ascending aortic dissections after aortic valve replacement.

DR. PENNY THOMAS (London, UK): Have you any idea from your specimens whether your bicuspid valves arose through the fusion of two leaflets early on in development, or whether there were always only two leaflets?

DR. BECHTEL: Most of them were noted in the surgical records to be congenitally bicuspid, which means that there are not always only two sinuses, but most of them were not fused as a result of a pathological process in adulthood.

DR. THOMAS: So the valves were like it when the patients were born?

DR. BECHTEL: That was the opinion of the operating surgeons. In this retrospective study I tried to include only those valves that appeared to be congenitally bicuspid.

DR. THOMAS: I am just trying to see if there is any link between any abnormality in the wall which might be neural crest-related or developmental with the number of leaflets, because neural crest cells do approach the leaflets.

DR. BECHTEL: I tried to include only those that were congenitally bicuspid.

DR. GAETANO THIENE (Padova, Italy): Did you have cases of aortic dissection with bicuspid valve? If so, was the aortic wall normal or abnormal?

DR. BECHTEL: We have cases with bicuspid aortic valves and dissection, but I can’t tell you at present what their pathology was like.
DR. THIENE: The problem is that it is impossible to dissect the aortic wall in the setting of a bicuspid valve without aortic wall abnormalities, because there is no hypertension there.

DR. BECHTEL: There were no dissections among the last eight patients I presented - they only had aneurysms.

Appendix I: Criteria for histological grading

Fibrosis
Grade 1: an increase in collagen content in an area comprising less than one-third of the total width of the media.
Grade 2: an increase in collagen in an area comprising between one- and two-thirds of the total width of the media.
Grade 3: an increase in collagen in an area comprising more than two-thirds of the total width of the media.

Atherosclerosis
Grade 1: intimal fibrous plaques, the thickness of which was less than one-fourth of the thickness of the media.
Grade 2: intimal fibrous plaques thicker than one-fourth of the media, or intimal plaques with minimal calcification and/or atheroma.
Grade 3: complex or complicated lesions of severe atheroma with thrombosis, calcifications and ulcerations.

Medionecrosis
Grade 1: focal loss of nuclei in an area comprising less than one-third of the total width.
Grade 2: focal loss of nuclei in an area comprising between one- and two-thirds of the medial thickness.
Grade 3: focal loss of nuclei in an area comprising more than two-thirds of the total medial thickness.

Cystic medial necrosis
Grade 1: minute foci of mucoid material (‘cysts’) were present within a single lamellar unit.
Grade 2: the amount of mucoid material had increased, so that accumulation of ‘cysts’ covered the total width of one lamellar unit.
Grade 3: the extent of mucoid material surpassed more than one lamellar unit, either because of focal accumulation of small ‘cysts’ within intact elastin lamellae or because of large ‘cysts’ in an area with fragmented elastin fibers.

Smooth muscle cell orientation
Grade 1: small foci with change in the orientation of the smooth muscle cells, which could be spread in different areas.
Grade 2: area with change in the orientation of the smooth muscle cell orientation or several areas that together represent between one-third and one-half of the thickness of the media.
Grade 3: large area of changes in the smooth muscle cell orientation, consisting more than one-half of the media thickness.

Elastic fragmentation
Grade 1: fewer than five foci with elastin fragmentation in one microscopic field (magnification $\times 200$), each focus comprising two to four neighboring elastin lamellae. The orientation of smooth muscle cells was preserved. Interruption of one elastin fiber alone was not interpreted as fragmentation.
Grade 2: five or more foci with elastin fragmentation in one microscopic field, each focus comprising two to four neighboring elastin lamellae. The foci could be confluent or scattered throughout the media. The orientation of smooth muscle cells was preserved.
Grade 3: presence of foci with elastin fragmentation in five or more neighboring elastin lamellae, irrespective of the number of foci per microscopic field. The smooth muscle cells showed alterations in orientation.

Inflammation
Grade 1: sparse scattered chronic inflammatory cells or an occasional small focus of inflammatory cells.
Grade 2: multiple small foci of inflammatory cells.
Grade 3: multiple large foci of inflammatory cells or a diffuse, heavy inflammatory cellular infiltrate.

*Based on data according to Schlatmann and Becker (13), Klima et al. (14) and de Sa et al. (10).