Ascending aortic aneurysm associated with bicuspid and tricuspid aortic valve: involvement and clinical relevance of smooth muscle cell apoptosis and expression of cell death-initiating proteins

Franz-Xaver Schmid\textsuperscript{a,}\textsuperscript{*}, Katrin Bielenberg\textsuperscript{b}, Anette Schneider\textsuperscript{c}, Andreas Haussler\textsuperscript{a}, Andreas Keyser\textsuperscript{a}, Dietrich Birnbaum\textsuperscript{a}

\textsuperscript{a}Department of Cardiothoracic and Vascular Surgery, University of Regensburg, Regensburg, Germany
\textsuperscript{b}Cardiovascular Research Unit, University Hospital Regensburg, Regensburg, Germany
\textsuperscript{c}Department of Anesthesiology, University Hospital Regensburg, Regensburg, Germany

Received 21 October 2002; received in revised form 21 November 2002; accepted 9 December 2002

Abstract

Objective: There is relationship between a dilated ascending aorta and a bicuspid aortic valve. Controversy exists concerning techniques available for surgical restoration of the functional and anatomical integrity of the aortic root. The present study was undertaken to define the histopathologic and molecular biologic condition of ascending aortic aneurysms associated with bicuspid (BAV) or tricuspid aortic valve (TAV) and the relationship to valve sparing or pulmonary autograft procedures.

Methods: Aortic aneurysm wall specimens from 20 patients (10 BAV; 10 TAV) undergoing elective repair and normal aortic tissues from organ donors (n = 5) were analysed for patterns of smooth muscle cells (SMCs) and infiltrating leukocytes (immunohistochemistry), apoptosis (in situ end-labelling of DNA-fragments (TUNEL)), and expression of the death-promoting proteins perforin, Fas, and FasLigand (Immunoblotting).

Results: Segments from aneurysms exhibited a distinct pattern of medial destruction, elastic fragmentation, and disorientation with rarefication of SMCs. BAV wall segments contained more cells bearing markers of apoptosis than TAV specimens whereas normal aorta displayed only few apoptotic cells ($P < 0.05$). TUNEL showed higher levels of DNA fragmentation in BAV than in TAV, and double immunostaining identified SMCs as the principal cell type displaying fragmented DNA. Immunohistochemistry confirmed expression of death-promoting mediators by infiltrating lymphocytes, and Western blotting documented their presence in BAV and TAV aneurysmal tissue, with the greatest increases seen in specimens from aneurysms associated with BAV.

Conclusions: There is evidence for a molecular link between SMC apoptosis initiated by infiltration and weakening of the aortic wall being more prevalent in patients with BAV. Our findings may suggest a mechanism responsible for aneurysm formation of the aorta and aortic dilatation after autograft root or sinus remodelling procedures.

Keywords: Aneurysm; Bicuspid aortic valve; Apoptosis

1. Introduction

A bicuspid aortic valve is among the commonest congenital heart valve abnormalities with a prevalence of approximately 1% in the general population [1]. The association of aortic wall complications such as anulooaortic ectasia, aortic dissection, spontaneous dissection of supraaortic arteries, and coarctation of the aorta have been well described [2,3]. Moreover, bicuspid aortic valve may result in premature calcific aortic valve stenosis or progressive aortic valve insufficiency and presents a risk factor for infective endocarditis. On the contrary, aortic root dilatation may be present with a functionally normal bicuspid aortic valve [4].

Some authors argued that aortic wall structural changes in that situation are predominantly the consequences of biomechanical stress [5,6] but others demonstrated that the pathologic conditions were also present in the absence of valve stenosis or incompetence. [3] Histopathological investigations of aneurysmal tissue have provided data

\textsuperscript{*} Presentated at the 16th Annual Meeting of the European Association for Cardio-thoracic Surgery, Monte Carlo, Monaco, September 22–25, 2002.

\textsuperscript{*} Corresponding author. Tel.: +49-941-944-9805; fax: +49-941-944-9802.

E-mail address: franz-xaver.schmid@klinik.uni.regensburg.de (F.X. Schmid).
concerning transmural inflammation and destruction of connective tissue [7,8]. Although these structural changes are considered characteristic in the pathophysiology of aneurysm formation, little information is available and the causal relationship remains undefined regarding mechanisms that might induce aneurysm development, growth and rupture.

The purpose of the present study was to evaluate histologic changes and molecular mechanisms in ascending aortic aneurysms associated with bicuspid or tricuspid aortic valve and to compare the features of degradation with those of normal aortas in order to gain further insights into the pathophysiology of aortic aneurysm formation.

2. Materials and methods

2.1. Aortic tissue

Full thickness aortic wall specimens were obtained from patients undergoing elective surgical repair for ascending aortic aneurysms associated with normal, tricuspid aortic valve (n = 10; eight men and two women, mean age 71.6 ± 9.0 years, range, 57–76 years), and ascending aortic aneurysms with a bicuspid aortic valve (n = 10; 12 men and eight women, mean age 59.8 ± 5.6 years, range, 42–70 years). The mean aneurysm size measured by computed tomographic (CT) scan and/or angiography for patients with BAV was 47.2 ± 5.6 mm (range, 35–54 mm), and for patients with TAV 56.1 ± 6.5 mm (range, 35–85 mm). For comparison, normal aortic wall segments were obtained from five organ donors, who had no evidence for aneurysmal or atherosclerotic disease, during multiorgan harvesting (average age 47.8 years, range, 31–57 years). Fresh tissue was divided and either immediately snap frozen in liquid nitrogen or paraffin embedded for immunohistochemistry, detection of apoptotic cells, and immunoblot isolation.

2.2. Immunohistochemistry

Aortic wall tissue specimens were analysed for monocytes/macrophages or lymphocytes and products of immune cells by immunohistochemistry using monoclonal antibodies. Paraffin-embedded sections (6 μm) were prepared and slide mounted. After rehydration and pre-treatment with target retrieval high pH solution (Dako) and 0.3% H2O2 to block endogeneous peroxidase, sections were incubated for 30 min in a blocking solution of 10% normal horse serum and then stained with primary monoclonal antibodies outlined in Table 1. After washing in Tris-buffered saline, incubation was performed with biotin-conjugated anti-mouse IgG antibody (Camon) for 60 min at room temperature. Bound antibodies were then recognised through avidin–biotin complex formation by an avidin–alkaline phosphatase fast reagent (Vectastain ABC kit, Vector). Normal mouse IgG (Sigma Chemical) served as the control for the immunostains.

Further differentiation was realised by double staining. First the TUNEL assay was performed, and then cell-specific antigens were determined by immunohistochemistry. In each section, 100 cells were examined by two independent investigators.

2.3. Detection of apoptosis by the TUNEL assay

Apoptosis is a form of cell death associated with cell shrinkage, chromatin margination, membrane blebbing and nuclear condensation. DNA fragmentation in apoptotic cells is followed by cell death. In order to quantitatively examine the occurrence of nuclear DNA fragmentation, we used the in situ end-labelling of DNA fragments (TUNEL) assay with an ApopTag detection kit (Intergen).

Principles of the procedure comprised formalin-fixed, paraffin-embedded tissue sections, rehydration and proteinase K pre-treatment (20 μg/ml). Incubation with 3.0% hydrogen peroxide provided quenching of endogenous peroxidase. Terminal deoxynucleotidyl transferase (TdT) enzyme was added to label DNA strands. After repeated washing anti-digoxigenin peroxidase conjugate was applied to the specimen and incubated. Nuclei staining was performed with 0.5% methyl green, and counterstaining

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Cell type</th>
<th>Source</th>
<th>Provider</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 3</td>
<td>Pan T cell</td>
<td>mma</td>
<td>Dako</td>
<td>1:20 IHC</td>
</tr>
<tr>
<td>CD 68</td>
<td>Macrophage</td>
<td>mma</td>
<td>Dako</td>
<td>1:50 IHC</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer cell</td>
<td>mma</td>
<td>Dako</td>
<td>1:20 IHC</td>
</tr>
<tr>
<td>CD 20</td>
<td>B cell</td>
<td>mma</td>
<td>Dako</td>
<td>1:50 IHC</td>
</tr>
<tr>
<td>HHF 35</td>
<td>Smooth muscle cell</td>
<td>mma</td>
<td>Dako</td>
<td>1:50 IHC</td>
</tr>
<tr>
<td>Perforin</td>
<td>T cells, macrophages</td>
<td>mma</td>
<td>T-cell Diagnostics</td>
<td>1:10 IHC</td>
</tr>
<tr>
<td>Fas</td>
<td>T cells, macrophages</td>
<td>mma</td>
<td>Pan-Vera</td>
<td>25 μg/ml IHC</td>
</tr>
<tr>
<td>FasL</td>
<td>T cells, macrophages</td>
<td>mpa</td>
<td>Transduction laboratories</td>
<td>1:2500 IB</td>
</tr>
</tbody>
</table>

Table 1
List of antibodies used for immunohistochemistry and immunoblotting in aortic tissue

* mma, mouse monoclonal antibody; mpa, mouse polyclonal antibody; ICH, immunohistochemistry; IB, immunoblotting
with peroxydase substrate diaminobenzidine (DAB) resulted in brown coloured condensed nuclei in apoptotic cells. Finally specimens were mounted and viewed under light microscopy.

2.4. Immunoblot analysis (Western blotting)

Vascular wall tissue samples were snap-frozen in liquid nitrogen, crushed and mixed with 0.5 ml of sodium dodecyl sulphate (SDS) protein extraction buffer (10% SDS, 20 mmol/l NaCl, 100 mmol/l Tris–HCl, pH 7.6). Centrifugation at 13 000 rpm was performed for 20 min at 4°C. After collection of the supernatant, protein concentration was determined using BSA as a standard.

For determination of activated Fas aggregate, a 7.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gel, otherwise a 12.5% SDS-PAGE minigel was used. The samples (30 μg protein/lane) were separated by electrophoresis for 1.25 h at 100 V. After transfer of the proteins to a membrane, the membranes were blocked with 5% low fat dried milk dissolved in Tris-buffered saline (TBS) (5 g per 100 ml) and incubated in TBS with primary antibodies against various T-cell antigens (Table 1) for 12 h at room temperature. After proper washing of the membranes with TBS, subsequent incubation in horseradish peroxidase (HRP)-labelled secondary antibody (anti-mouse IgG) was performed for 1 h. The washed membranes were analysed with a light emitting non-radioactive enhanced chemiluminescence system (ECL™, Amersham).

Fig. 1. Representative sections of ascending aortic aneurysmal tissue incubated with murine anti-α-actin antibody. Rarefication of smooth muscle cells in aneurysmal tissue. (a) Aneurysm in patient with tricuspid aortic valve TAV; (b) high power view of region indicated in a; (c) aneurysm in patient with bicuspid aortic valve BAV; (d) high power view of region indicated in c; low power magnification × 10, high power magnification × 40.
2.5. Statistical analysis

During light microscopy, ten adjacent fields of each section were analysed, and the counts of two independent investigators were averaged. Apoptotic index (AI) was calculated according to the formula for total cells [9]:

\[
AI = \frac{100 \times \text{(number of TUNEL-positive cell nuclei)}}{\text{total number of nuclei}}
\]

and for SMCs:

\[
AI = \frac{100 \times \text{(number of TUNEL-positive cell nuclei)}}{\text{\(\alpha\)-actin-positive SMCs}}
\]

Data are presented as means ± standard deviation. Significant differences between means were determined by Student’s \(t\)-test. Statistical significance was set at \(P < 0.05\).

3. Results

3.1. Histologic features

The principal finding of the study was that degenerative and inflammatory changes were present in aneurysmatic aortic tissue of patients with bicuspid and tricuspid aortic valves, but appeared to be more severe in patients with BAV. In normal aortic media, almost all smooth muscle cells (SMCs) showed immunoreactive \(\alpha\)-actin and formed arrangement in a well-organised pattern of elastic laminae. In contrast, aneurysmal aorta exhibited fewer actin-positive cells interspersed in hypocellular areas. In regions with relatively high cell density, cells were fragmented or disrupted (Fig. 1). To eliminate influences of regional differences in cellularity and of magnification, quantification of \(\alpha\)-actin levels is expressed as percent of values for healthy aorta. Calculation of the numbers of nuclei per cross-sectional area demonstrated a 25% decrease in the nuclei per unit area in TAV aneurysms, and a 32% decrease in BAV tissue, respectively, when compared to normal aorta (\(P < 0.01\)).

### Table 2

<table>
<thead>
<tr>
<th>Staining</th>
<th>Control (range)</th>
<th>TAV (range) (%)</th>
<th>BAV (range) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)-Actin</td>
<td>97% (93–99%)</td>
<td>54 (34–66)</td>
<td>65 (44–75)</td>
</tr>
<tr>
<td>CD3</td>
<td>&lt;1%</td>
<td>17 (12–20)</td>
<td>10 (4–18)</td>
</tr>
<tr>
<td>CD68</td>
<td>5%</td>
<td>16 (2–22%)</td>
<td>15 (8–32)</td>
</tr>
<tr>
<td>CD20</td>
<td>&lt;1%</td>
<td>5 (1–10%)</td>
<td>4 (0–10)</td>
</tr>
<tr>
<td>NK</td>
<td>&lt;1%</td>
<td>2 (0–6)</td>
<td>2 (0–4)</td>
</tr>
</tbody>
</table>

\(\alpha\)-Actin, smooth muscle cell; CD3, Pan T cell; CD68, macrophage; CD20, B cell; NK, natural killer cell; BAV, bicuspid aortic valve aneurysm; TAV, tricuspid aortic valve aneurysm.

3.2. Immunohistochemistry

Immunohistochemistry was used for determination of monocytes/macrophages or lymphocyte accumulation in aneurysmal tissue. Only few CD3 + T cells or CD68 + macrophages were recognised in normal control aorta. In aneurysmal tissue, there was clear evidence of macrophage, T lymphocyte, and to a minor extent, B lymphocyte and natural killer cell infiltration. The cumulated results of all three groups of specimens are shown in Table 2. Antibody staining characterised 17% of cells in TAV tissue and 10% in BAV tissue as T cells, and 16% of cells in TAV tissue and 15% in BAV tissue as monocytes/macrophages, respectively. There were no major differences between wall specimens obtained from patients with TAV and BAV with respect to subsets of infiltrating leukocytes.

3.3. Markers of apoptosis

The significant decrease in the number of SMCs, especially in aortic walls of BAV patients, led us to assume programmed cell death to be responsible for SMC rarefication. DNA fragmentation detected by TUNEL technique served as an apoptotic marker. In the media of normal aortic tissue, there were only very few TUNEL positive cells (<1%). In contrast, sections of aneurysms and particularly of aneurysms from patients with BAV, depicted numerous TUNEL positive cells. Double staining by TUNEL and immunohistochemistry enabled to differentiate cell types bearing markers of apoptosis. Cells demonstrating TUNEL positive nuclei were predominantly SMCs. Determination of the apoptotic index (ten adjacent high power fields, two independent observers) displayed significantly increased numbers of dead SMCs in aneurysmal sections (TAV: \(AI = 9.7 \pm 3.6\); BAV: \(AI = 10.9 \pm 5.2\)) when compared to normal aorta (\(AI = 1.2 \pm 0.8\)) (Fig. 2).

![Fig. 2. Calculated apoptotic index (AI) determined by light microscopy for all cells by counting of cells with evident condensed bodies in nucleus and for smooth muscle cells (SMCs) only by additional \(\alpha\)-actin-staining (Normal, normal aorta; BAV, aneurysm with bicuspid aortic valve; TAV, aneurysm with tricuspid aortic valve).](image-url)
To explore mediators of cell death produced by infiltrating immune cells, we evaluated levels of candidate mediators (Fas, perforin) in extracts of aortic tissue by immunohistochemistry and immunoblotting. The normal aorta exhibited Fas antigen neither in the intima nor in the media whereas in tissue sections derived from aneurysm resections there was an increased expression of the Fas molecule both in SMCs and leukocytes. In accordance to the histologic findings, only extracts from diseased aorta, BAV and TAV aneurysms, demonstrated presence of the death-promoting proteins Fas as well as of perforin. Specimens from all patients were examined by Western blot analysis. The results for some representative cases are demonstrated in Fig. 3.

3.4. Mediators of cell death

To explore mediators of cell death produced by infiltrating immune cells, we evaluated levels of candidate mediators (Fas, perforin) in extracts of aortic tissue by immunohistochemistry and immunoblotting. The normal aorta exhibited Fas antigen neither in the intima nor in the media whereas in tissue sections derived from aneurysm resections there was an increased expression of the Fas molecule both in SMCs and leukocytes. In accordance to the histologic findings, only extracts from diseased aorta, BAV and TAV aneurysms, demonstrated presence of the death-promoting proteins Fas as well as of perforin. Specimens from all patients were examined by Western blot analysis. The results for some representative cases are demonstrated in Fig. 3.

4. Discussion

Pathology studies have clearly demonstrated an association between bicuspid aortic valve and aortic medial abnormalities [8,10]. Our group examined the degree of smooth muscle cell rarefaction, leukocyte infiltration, and expression of cell death-initiating proteins at the tissue level in ascending aortic aneurysms of patients with bicuspid and tricuspid aortic valve. The study confirmed that patients with congenitally bicuspid aortic valve have more severe inflammatory as well as degenerative changes in the medial layer than patients with tricuspid aortic valve disease. Although there was no difference in infiltrating leukocyte subsets, patients with a bicuspid aortic valve had fewer numbers of SMCs depicted by a significantly increased apoptotic index for all medial cells, but especially for SMCs. Another important finding of our study was the demonstration of death-promoting mediator expression, perforin and Fas/FasL, in sections and extracts of BAV and TAV aneurysmal tissue but not in normal aorta.

After a decade of discussion concerning concepts of the pathogenesis of aneurysm formation in the ascending aorta, there is nowadays evidence of an active disease process besides mechanical or tensile stress [5,6] or genetic predisposition [11]. The fact that a minority of patients with congenitally bicuspid aortic valve do not develop valvar disease or ascending aortic dilatation [3,12] implicates that factors other than biomechanical stress might be responsible for structural changes. Aneurysms have been traditionally considered a manifestation or complicated form of atherosclerosis. Aortic aneurysms are clearly associated with accelerated degradation of aortic wall structural components. Not surprisingly, we found breakdown of elastic laminae and disappearance of well-organised smooth muscle layers, non-specific markers of degenerative aortic wall disease, as histologic features in aneurysmal tissue of BAV and TAV patients. These pathologic changes were found to be similar to those described in patients with aortic dissection, ascending aortic aneurysms, or anuloaortic ectasia [13–15]. Others documented patients with bicuspid aortic valve to have thinner and more distant elastic lamellae of the media than patients with a tricuspid aortic valve using morphometric analysis [7,8]. So far, our findings confirm the importance of this aspect in aneurysm disease and are also in accordance with previous reports [16,17].

During the past few decades, evidence for chronic inflammation as a common finding in human aortic aneurysms has accumulated. In this study, histological examination and quantitative grading of leukocyte infiltration in sections derived from patients with BAV and TAV showed T lymphocyte and macrophage infiltration with no major difference between the two in the extent and localisation of the infiltrates. Newman et al. [18] argued that inflammatory cell products within degenerative media of aneurysms may play a role in the destruction of connective tissue proteins. A loss or enzymatic breakdown causes reduced strength and elasticity to the arterial tunica media. Controversy exists regarding the impact of metalloproteinases (MMPs) and their inhibitors (TIMPs) on histologic changes [19]. Tung and associates [20] have found MMP-9 to be elevated at both the protein and mRNA levels in aneurysm disease. Others [21] have described that TIMP-1 is also expressed in aneurysmal tissue as well as in normal aorta. Currently findings by Gregory et al. [22] have led to speculation that a specific immune response, autoimmunity in aortic aneurysm, might contribute to this
Another limitation is that we examined tissue specimens of mental stages of aneurysm formation from our observations. End-stage disease but we cannot conclude earlier development from aortic tissue derived from BAV patients in comparison to aortic aneurysms demonstrating more severe changes in inflammatory infiltration and SMC apoptosis play a role in promoting products of activated immune cells in thoracic aorta. Consistent with findings on human abdominal aortic aneurysmal tissue whereas non-aneurysmal aortic tissue contained only few TUNEL-positive cells. Cells bearing this marker of apoptosis were preferably colocalised adjacent to inflammatory infiltrates. Thus aneurysms in BAV patients show a similar histologic picture to TAV patients with respect to leukocyte infiltration, but differ in the degree of SMC apoptosis. Nevertheless, this finding suggests local initiation of cell death by mediators produced by infiltrating immune cells in aneurysmal tissue.

Apoptosis is initiated by activation of a cascade of signals including the death-promoting mediators Fas and perforin. Fas, a member of the tumor necrosis factor (TNF) receptor family, has been identified to be involved in T cell-mediated cytotoxicity [24]. By binding to its ligand FasL, Fas is capable to start cytoplasmatic signaling cascades that can lead to cell death. Particularly T cells are also responsible for the release of perforin molecules that can attack cell membranes and ultimately kill target cells. Our observation of inflammatory infiltration of leukocytes and expression of death-promoting mediators in BAV and TAV aneurysm sections but not in non-aneurysmal tissue provides biochemical evidence for the role of activated T cells producing FasL and perforin to induce SMC apoptosis. To our knowledge, there are no molecular biological observations on aneurysmal tissue derived from thoracic aorta. Consistent with findings on human abdominal aortic sections [9], we found pronounced expression of death-promoting products of activated immune cells in thoracic aortic aneurysms demonstrating more severe changes in aortic tissue derived from BAV patients in comparison to specimens from TAV patients.

The principal limitation of this descriptive and comparative study is that our analyses were made on only a few sections from a representative portion of surgically removed wall segments. The study can only determine whether inflammatory infiltration and SMC apoptosis play a role in end-stage disease but we cannot conclude earlier developmental stages of aneurysm formation from our observations. Another limitation is that we examined tissue specimens from different age groups. It remains unclear whether age contributed to the activity and extent of inflammatory and degenerative processes.

5. Conclusion

Although the disease process in bicuspid and tricuspid valve patients appeared similar and because most patients with a bicuspid valve eventually need surgery, these findings have important clinical implications. Only about 1% of patients maintain normal valve function over their lifetime [3]. Severe structural changes in the aortic media lead to reduced strength of the aortic wall and account for an increased incidence of aortic root and tube dilatation. In light of currently available surgical techniques with good immediate and long-term results and minimal risk in elective operations [4] more liberal indications for surgery of the dilated ascending aorta are advocated. We recommend external fixation, for instance, by strips or rings of Dacron fabric, during reconstructive surgery of the aortic root when autologous tissue is left in place. An ongoing controversy concerning the correlation of aortic valve structure and late autograft dilatation [10,17] and the fact of a common embryologic origin of the aortic and pulmonary roots, the conotruncus [11,25], may even support this strategy during autograft replacement procedures. Aggressive evaluation and treatment of conventional atherosclerotic risk factors are indicated especially in patients with a bicuspid aortic valve. Based on our understanding of the disease process at the molecular level and on the knowledge of clinical associations an effective treatment, hopefully available in the near future, should begin at the time of diagnosis, delaying or even avoiding the need for valve and/or aortic surgery.

References

[8] Parai JL, Masters RG, Walley VM, Stinson WA, Veinot JP. Aortic...

Appendix A. Conference discussion

Dr H. Borst (Munich, Germany): I just wonder why you did not do electronmicroscopy, because most of the work on the aortic wall, of course, has been done that way and it might have been nice to compare it.

Dr Schmid: Actually the work is very time-consuming and also cost-consuming. This is one fact. The other fact is we tried to have any conjunction with biochemical or pathological processes. So the way to analyze the expression or the release of proteins on the protein level is absolutely a new way, and as I tried to point out, it was our primary interest to see any mechanistic link between inflammation, infiltration and degeneration. Through electronmicroscopy you have just the documentation of an end stage of the disease, but we are interested in the future in the developmental processes of aortic aneurysms, because it is maybe possible to deduce some therapeutic approaches from those findings.

Dr Borst: I certainly agree that it would be interesting to look at other aortic diseases with the same method in the future.

Now, as far as clinical relevance is concerned, you didn’t suggest that bicuspid valves should be sacrificed, did you, during operation?

Dr Schmid: If you have to do surgery on a patient with an ascending aortic aneurysm and you find a functionally normal bicuspid aortic valve, I think it is not justified to do anything with it.

Dr Borst: I didn’t quite agree with the idea that you have to do an external wrap. Your results are grand dad surgery, actually. I think if you want to eliminate the ascending aorta, you do a Tirone David I procedure and that takes care of that regardless of the morphology of the valve.

Dr Schmid: Yes, that’s right. Actually maybe this is the limitation of the study. The specimens were taken from the ascending aorta, and usually the dilatation after surgical restoration of the root occurs in the region of the sinuses, though we cannot clearly say that the process of degeneration, or whatever, in the root is the same as in the ascending aorta.

Dr J. Roquette (Lisbon, Portugal): Did you find any relation with age in these patients?

Dr Schmid: We didn’t try to do it because, as you have seen, the number of patients was very limited, it was just 10 patients in one group because of the time- and work-consuming procedures we have performed, and the age of the patients was more or less comparable, though there were no significant differences. They were all in the age of 50 to 70 except the organ donors, who were slightly younger.

Dr F. Maisano (Milan, Italy): Did you find any correlation between the diameter of the aneurysm and the grade of inflammatory disorders you found?

Dr Schmid: This is more or less the same question I just answered. Inflammatory processes are very active in young persons and the older the patient will be the less severe will be the inflammatory process. But we didn’t do a quantification analysis concerning the age of the patients or the size of the aneurysm. We just compared the patients with aneurysms, and the definition was above 5 cm in diameter with a bicuspid and a tricuspid aortic valve. It just happened that patients came in to be operated.

Dr Maisano: The question is whether the inflammation is the primary cause or is a secondary effect of the dilatation.

Dr Schmid: I can’t answer whether it is primary or secondary. We just described a situation that you have inflammation. It has been described in a number of vascular diseases. But this is clearly a contrary situation, for example, to vascular occlusive disease where you have no inflammation; you have just degradation.