Comparison of biomechanical properties in ascending aortic aneurysms of patients with congenital bicuspid aortic valve and Marfan syndrome

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Mechanistic Interrogation of Bicuspid Aortic Valve associated Aortopathy (MIBAVA) Leducq Consortium: 1

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ABSTRACT

Background: In patients with ascending aortic aneurysms (AscAA), biomechanical differences are seen among patients with congenital bicuspid aortic valves (BAV), Marfan syndrome (MFS), and tricuspid aortic valves (TAV). We examined the hemodynamic profiles and ultrastructures of aneurysmal specimens, focusing on vascular remodelling to better understand AscAA pathogenesis.

Methods: A total of 795 patients with BAV (43.97 ± 0.51 years; 93.2% male), 69 with MFS (34.43 ± 1.44 years; 86.2% male), and 90 with TAV (67.27 ± 0.58 years; 60% male) were enrolled, primarily upon admission with AscAA. The biomechanical properties of the aortic root were assessed and intraoperative specimens were analyzed by light-microscopy and two-photon autofluorescence microscopy.

Results: Patients with BAV had significantly greater distension of the aortic root, irrespective of age or aneurysmal widening ($R^2 = 0.543, p < 0.05$). This was associated with significantly increase in the size of the tunica media. Patients with MFS displayed significant stiffness in the sinuses that worsened with age ($R^2 = 0.752, p < 0.001$), similar to patients with TAV ($R^2 = 0.626, p < 0.05$). Patients with MFS showed significant root elasticity with aneurysmal growth ($R^2 = 0.596, p < 0.05$) and increased medial degeneration. Patients with TAV maintained biomechanical properties, apart from aneurysmal dimensions and high levels of inflammation.

Conclusions: Among patients with AscAA, those with BAV maintain tissue elasticity in the aortic root, regardless of age. Patients with MFS demonstrate increased sinus stiffness with medial degeneration, both during aging and with aneurysmal growth. Patients with TAV and AscAA present with increased inflammation.

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1. Introduction

The normal thoracic ascending aorta has a strict architecture with respect to elastic fiber quantity, quality, and organization as well as surrounding matrix conditions [1]. This architecture is often altered in connective tissue disorders such as Marfan syndrome (MFS) and the non-syndromic bicuspid aortic valve (BAV). While the pathogenesis of MFS and relevant ascending aortic aneurysms has been well studied [2,3], the precise mechanisms underlying the development and progression of a thoracic aortic aneurysm in BAV patients remain remarkably elusive [4–6]. BAV, the most common congenital heart malformation, presents a wide range of heterogeneous morphological phenotypes of fused cusps and associated aortopathies [7–9]. Early studies of BAV reported microscopical degenerations such as cystic medial necrosis, elastin fragmentation, and loss of smooth muscle cells as well as their orientation in aneurysmal walls [10]. Though, other studies have explored the evidence of fewer severe inflammation abnormalities in BAV patients compared with those with tricuspid aortic valves [11–13]. The current grading of BAV-induced aortic abnormalities in the histopathological evaluation is based on conflicting reports. These diverse findings hint at the complexity of BAV-associated aortopathy. So far, two popular theories have been proposed to explain the development of aortic dilatation associated with BAV: (1) dilatation is accompanied by constant hemodynamic stress because of a varied flow profile through the diseased valve, which is commonly assessed in valve stenosis, and (2) aneurysmal development is associated with congenital aortic fragility, which is responsible for progressive matrix degeneration and corresponding vascular remodeling in the ascending aorta. However, interpreting hemodynamic derangements is comparable to “seeing one spot on a leopard” when trying to understand clearly the pathogenesis of aortopathy.

In this study, we defined the sizes of the aorta using multislice computed tomography with further quantification based on individual body surface area (BSA) in square meters. Both the elastic properties of the aortic root and ascending aorta and the histological characteristics of aneurysms were evaluated and compared.

2. Materials and methods

2.1. Cohort

From January 1, 2002, to December 31, 2016, we enrolled 954 patients (48.56 ± 0.84 years, 79.8% male) with primary thoracic ascending aorta dilatation or dilation on admission. All patients underwent Elective cardiac operations. In this cohort, there were 795 patients with congenital BAV (43.97 ± 0.51 years; 93.2% male), 69 with MFS (34.43 ± 1.44 years; 86.25% male), and 90 with TAV (67.27 ± 0.58 years; 60% male). The TAV group was defined as the control. To generate an appropriate comparison, we carried out propensity score matching (PSM) to improve the comparability of the groups.

2.2. Materials

We collected the aortic resections intraoperatively. Samples were obtained for histopathological analysis from the concave and convex aortic sites and the proximal and distal aneurysmal sections, respectively. Tissue samples were fixed in a solution of 4.5% formalin buffer, pH 7.4, at 4 °C followed by routine procedures. The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from each patient.

2.3. Methods

We arranged all patients for electrocardiogram-gated multislice thoracic computed tomography (Siemens Symbia Intevo; slice thickness = 1 mm, kV = 120, mA = 500, mA K = 111) upon admission to evaluate the size of the ascending aorta at different levels. Thoracic ascending aneurysms were quantitatively assessed for individual BSA features in square meters (m²) according to the method of Dubois [14]. Aortic strain, aortic distensibility (DIS), and stiffness index (SI) were measured by echocardiographic M-mode of the root with simultaneous measurement of blood pressure by cuff sphygmomanometer and calculated using the following equations:

$$\text{Distensibility} = \frac{A_t - A_d}{A_d} \times \frac{10^3}{(P_s - P_d) \times 333} \text{ (kPa}^{-1} \text{ kPa}^{-3})$$

$$\text{Stiffness Index} = \frac{P_s}{P_d} \times \frac{D_d}{D_s} \text{ (dimensionless)}$$

$$\text{Strain stress} = \frac{(D_s - D_d)}{D_d} \times 100 \text{ (dimensionless)}$$

where As is area in systole, Ad is area in diastole, Ps is systolic pressure, Pd is diastolic pressure, Ds is diameter in mid-systole, and Dd is diameter in mid-diastole.

2.4. Statistical analysis

Data were analyzed using the SPS for Windows (version 18.0, PASW; IBM). Continuous data were expressed as mean ± standard error and with a range where appropriate. Nominal data were expressed as numbers and percentages. Means per group were compared using the one-way ANOVA. Categorical variables were analyzed using the χ² test, t-test, Levene’s test, and regression method. In this study, a logistic model was defined using PSM for matching. Statistical significance was set at a two-tailed probability value of p < 0.05.

3. Results

3.1. Macroscopic morphological parameters

The BAV group showed more ascending dilatation (22.92 ± 1.42 mm/m²) compared with the MFS group (21.14 ± 1.50 mm/m²) and TAV group (21.98 ± 0.26 mm/m²) (F(2) = 67.408; p < 0.05), while the MFS group had significant predominance in the aortic sinuses (p < 0.001; Table 1). However, the BAV group proved such special morphological patterns as “annulus < STJ < sinus ≪ ascendants”, whereas the MFS group had showed “annulus < STJ < ascends ≪ sinus”. The TAV group performed an equivalent, but slightly constricted, outline of aortic ascends as the BAV group did. We found a significant association between groups and aortic valves disease, but the strength of association was moderate (χ² [3, N = 954] = 199.767, p < 0.001, Phi = 0.502).

3.2. Biochemical parameters

The BAV group showed significantly more distensibility and strain at the annulus level in the aortic root compared with the MFS and TAV groups (distensibility: 48.63 ± 1.08 vs. 28.21 ± 1.84 vs. 21.95 ± 0.92 kPa−1 · 10−3; strain: 16.47 ± 0.35 vs. 8.35 ± 0.73 vs. 12.63 ± 0.67; F(2) = 41.799, p < 0.001), while significantly more stiffness was presented in the aortic sinuses in patients with MFS (12.30 ± 0.62 vs. 4.29 ± 0.14 vs. 8.47 ± 0.21; F(2) = 236.554, p < 0.001). Patients with BAV demonstrated biomechanical characteristics such as distensibility and aortic strain maintained more in the lower part of root (distensibility_annulus 48.63 ± 1.08 > distensibility_sinum 39.87 ± 0.93; strain_annulus 16.47 ± 0.35 > strain_sinum 10.81 ± 0.43) (Table 2).

This phenomenon persisted over the years in terms of aging in this group. However, patients with MFS lost distensibility progressively in root structures during aging (R² = 0.731, p < 0.001), accompanied with progressive stiffness of vessel walls, as the ones with TAV did (R² = 0.626, p < 0.05). Furthermore, with increasing of ascending aneurysm dimension, patients with MFS lost the distensible property in the root structures, where the lower part declined precipitously (R² = 0.596, p < 0.05). On the contrary, patients with BAV had the compliance of...
the lower root even increased with increasing diameters of ascending aneurysms ($R^2 = 0.543$, $p < 0.05$). Accordingly, the annulus bears increasing stress with aneurysmal enlargement ($R^2 = 0.649$, $p < 0.01$) in this group. However, the biomechanical properties kept stable quite apart from aneurysmal dimensions in the TAV group (Fig. 1).

### 3.3. Microscopic histological parameters

The BAV group presented the largest distribution of the medial layer in terms of the percentage of thickness of tunica media to the whole vessel wall (80.63% ± 1.26%) in the aneurysmal wall. By contrast, the TAV group had the thinnest tunica media (47.29% ± 0.36%) ($F_{(2)} = 127.038$; $p < 0.001$) (Fig. 2).

Medial degenerations such as mucoid extracellular matrix accumulation, laminar medial collapse in the sense of cystic medial necrosis, elastic fiber fragmentation, loss, or disorganization of smooth muscle cells, medial fibrosis with an increase in collagen layers, and decreased inflammatory change were less observed in the aneurysmal specimens of patients with BAV. But their elastic fibers were much more directionally oriented and densely compacted in bundles with continuity ($p < 0.001$). However, more medial degenerative changes, such as cystic medial necrosis, smooth muscle cell disorientation, and elastic disarrangement were observed in patients with MFS than the others ($p < 0.001$). Patients with TAV often showed medionecrosis, atherosclerosis, and infiltration of inflammatory cells in sense of inflammatory changes ($p < 0.001$). Regardless of age-related changes in the arterial vessels, such as thickening of intima through eccentric and or diffuse fibrous rearrangement, the adventitia is the thickest layer of the aneurysmal wall with extensively scattered fibillary collagen bundles in sense of increased fibrosis and some lymphocyte infiltrates. Within the tunica media, the elastic fibers are loosely cross-linked with supplemented collagen ingredients (Fig. 2).

### 4. Discussion

Thoracic aortic aneurysms develop because of vascular remodeling, which leads to weakness of the aortic ultrastructures and final dilatation. Such dilatation should be considered as a part of the aortoventricular complex, which comprises the left ventricle, the aortic valves, the root, and the vessel wall. Each part of this complex can impair the others. This induces a dysfunction at multiple levels in the sense of aortopathy. Vascular remodeling is a homeostatic response to flow change and circumferential expansion in a recoiling manner to restore shear stress and vessel wall tension. These behaviors of the aortic vessel wall are determined by individual biomechanical properties forcing blood flow along the root and ascending aorta [16]. Hemodynamics undoubtedly contribute to aortic dilatation. Aortic elasticity features such as distensibility are essential to preserve root function and adjust the ejecting flow profile. As shown in this study, patients with BAV demonstrated improved root tensile power in the descending flow direction, and this phenomenon remained stable over the years. MFS patients experienced progressive loss of root distensibility during the aging process in terms of increased stiffness, whereby there was a mild shift in strain-stress from the lower to the upper part of the aortic root. The same was observable even more distinctly in the TAV group, with the exception of an increase over the years in strain-stress load in the upper portion of the root. This suggests that vascular remodeling in the form of progressive calcification could speed up with cardiac remodeling.

On the other hand, the aorta’s elasticity and tensile strength come from its medial structures such as the lamellae of elastin, collagen, and vascular smooth muscle cell [17]. The aortic aneurysms are histologically characterized by media thinning caused by proteolytic injury to the extracellular matrix and vascular smooth muscle cell degeneration with relevant collagen network remodeling. Such a disorder worsens in a vicious cycle towards aortic dilatation and rupture. We observed that the MFS group presented greater medial degeneration.

### Table 1

Evaluation of physical parameters and dimensions of thoracic aortic ectasia and aneurysms.

<table>
<thead>
<tr>
<th></th>
<th>MFS group (n = 69)</th>
<th>BAV group (n = 795)</th>
<th>TAV group (n = 90)</th>
<th>F(df = 2); p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.43 ± 1.44</td>
<td>43.97 ± 0.51</td>
<td>67.27 ± 0.58</td>
<td>158.989; &lt;0.001</td>
</tr>
<tr>
<td>Gender (male:female)</td>
<td>46:23</td>
<td>647:148</td>
<td>54:36</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.59 ± 0.43</td>
<td>26.20 ± 0.23</td>
<td>27.13 ± 0.18</td>
<td>38.079; &lt;0.001</td>
</tr>
<tr>
<td>BSA (DuBois m²)</td>
<td>2.07 ± 0.03</td>
<td>2.00 ± 0.01</td>
<td>1.93 ± 0.01</td>
<td>0.167; 0.683</td>
</tr>
<tr>
<td>AoV-vitium</td>
<td>No vitium</td>
<td>25.4%</td>
<td>0.8%</td>
<td>7.6%</td>
</tr>
<tr>
<td></td>
<td>Pure AoV stenosis</td>
<td>-</td>
<td>24.7%</td>
<td>18.4%</td>
</tr>
<tr>
<td></td>
<td>Pure AoV regurgitation</td>
<td>73.1%</td>
<td>32.0%</td>
<td>38.9%</td>
</tr>
<tr>
<td></td>
<td>Combined AoV vitium</td>
<td>1.5%</td>
<td>42.6%</td>
<td>35.1%</td>
</tr>
<tr>
<td>Aortic sections (mm)</td>
<td>Annulus</td>
<td>32.21 ± 1.19</td>
<td>27.07 ± 0.21</td>
<td>27.72 ± 1.58</td>
</tr>
<tr>
<td></td>
<td>Sinus</td>
<td>56.11 ± 1.40</td>
<td>35.96 ± 0.32</td>
<td>38.52 ± 1.47</td>
</tr>
<tr>
<td></td>
<td>STJ</td>
<td>41.29 ± 1.78</td>
<td>31.10 ± 0.31</td>
<td>32.04 ± 1.65</td>
</tr>
<tr>
<td></td>
<td>Ascendens (max.)</td>
<td>42.67 ± 2.44</td>
<td>45.36 ± 2.68</td>
<td>41.94 ± 0.46</td>
</tr>
<tr>
<td>Aortic sections relative to BSA (mm/m²)</td>
<td>Annulus/BSA</td>
<td>15.39 ± 0.62</td>
<td>13.64 ± 0.11</td>
<td>14.47 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>Sinus/BSA</td>
<td>27.26 ± 0.72</td>
<td>18.07 ± 0.15</td>
<td>19.88 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>STJ/BSA</td>
<td>19.84 ± 1.00</td>
<td>15.64 ± 0.15</td>
<td>16.72 ± 0.86</td>
</tr>
<tr>
<td></td>
<td>Ascendens (max.)/BSA</td>
<td>21.14 ± 1.50</td>
<td>22.92 ± 1.42</td>
<td>21.98 ± 0.26</td>
</tr>
</tbody>
</table>

Abbreviations: BMI = body mass index; BSA = body surface area; AoV-vitium = aortic valve vitium; STJ = sinotubular junction.
which comprehensively weakened the compliance. In this group, the
distensibility in the root was lost with the aneurysmal growth, especially
in the lower part of the root, suggesting generalized tissue fragility that
was reasonable for the pathogenesis. However, patients with congenital
BAV presented with fewer degenerative disorders in the aneurysmal
specimens, where elastic fibers were much more directionally oriented
and densely compacted in bundles with continuity (Fig. 2). Weighted
by the aneurysmal diameter, the BAV group demonstrated distensibility
in the lower root that increased with aneurysmal growth. A large com-
pliance reservoir in the root structures in the BAV group is plausible.
Moreover, the tissue specimens of patients with TAV exhibited signi-
ficant inflammatory changes. While elasticity-related features, such as
compliance and stiffness, were adversely affected by aging, these prop-
erties remained stable as the aneurysm diameter increased.

It is still debated whether patients with connective tissue disorders
in whom the ascending aorta and root are of borderline size should
undergo early treatment. Here, therapeutic interventions depend on the
cause. Actually, some decisions about a suitable therapeutic procedure
are often made by the operating surgeons intraoperatively on site. The
treatment decision depends not only on the surgeon’s experiences but
also on the insights into the underlying pathogenic mechanisms. Since
most patients were young adults in this study, we preferred procedures
that could maintain root compliance and provide valves with adequate
motion behavior. Thus, two methods were available: remodeling of the

![Fig. 1. Biomechanical properties such as distensibility, stiffness index, and aortic strain were assessed at different levels of the aortic root.](image1)

![Fig. 2. A general representative view of the vessel walls of the aneurysmal specimens is presented using light microscopy (upper row, 60×), while analysis of elastic fibers and collagen components in the tunica media were taken using autofluorescence and second-harmonic generation based two-photon microscopy at an excitation wavelength of 780 nm and 1100 nm (lower row), respectively.](image2)
aortic root with supravalvular annuloplasty and reimplantation of the aortic valves with sub- and supravalvular annuloplasty. Each method has advantages and limitations. Based on the technical requirements, a hybrid procedure such as remodeling with an external subvalvular aortic ring annuloplasty is rational [18]. Nevertheless, it is difficult to ignore the inevitability of using the Ross procedure under these circumstances, when the aortic valve cusps are irreparable. However, surgical treatment should be considered only for subjects who have aneurysm diameters close to the pathological reference. Therefore, therapeutic treatment with, for example, the reimplantation technique is preferred in patients with MFS but is avoided in patients with BAV. A further therapeutic option that uses anti-inflammatory agents can be considered for patients with normal TAV. In view of the particular morphological and biomechanical patterns of the aneurysm in patients with BAV and MFS, both special therapeutic strategies and targeted follow-up with clinical controls are necessary. Future prospective studies with long-term evaluation of vascular remodeling and rearrangement of biomechanical properties are needed to gain more understanding of the underlying pathogenesis.

5. Study limitations

With regard to biomechanical properties of the aneurysmal aorta in our patients, the measurement of blood pressure in this study was not performed invasively in the aorta but rather using cuff sphygmonanometry of the brachial artery. This is because earlier studies have shown that functional indices of the aorta obtained noninvasively are appropriately correlated with those achieved from aortography [19]. However, a similar correlation was observed between indices drawn from noninvasive methods in terms of brachial artery pressure and those using central pulse pressure captured by artery tonometry and pulse wave analysis. However, in our study, the noninvasive method of transthoracic echocardiography disappointingly limited hemodynamic assessment in the tubular portion of the ascending aorta because of limits in its technical accessibility in some individuals. Although the aortic valve functionality is a determinant of the flow profile, it was not thoroughly discussed in this study owing to the complexity of valvular effects in turbulence formation without stratification using valve diseases.

This study was designed to assess the interplay between morphology and biomechanical properties with and without the onset of aneurysmal formation in the ascending thoracic aorta in patients with congenital BAV, MFS, and TAV. General abnormalities in biomechanical characteristics were compared, irrespective of the growth rate of aortic dilatation observed in patient history. Unfortunately, in this retrospective study, the number of patients in the TAV control group was limited owing to the qualitative restriction of the aortic samples, which are rare. We used PSM to establish a result that can be used for comparing variables of interest, whereas statistical properties of PSM exhibited low standards. These properties were estimated by running a logit model with a binary outcome variable. Few covariates related to aneurysmal pathogenesis and development were considered for the matching process.

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Disclosures

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