Intrinsic Histological Abnormalities of Aortic Root and Ascending Aorta in Tetralogy of Fallot
Evidence of Causative Mechanism for Aortic Dilatation and Aortopathy

J.L. Tan, MBBS, MRCP; P.A. Davlouros, MD; K.P. McCarthy, BSc; M.A. Gatzoulis, MD, PhD; S.Y. Ho, PhD, FRCPa

Background—Dilatation of the aortic root is a known feature in tetralogy of Fallot (TOF) patients with pulmonary stenosis (PS) or pulmonary atresia (PA). We hypothesized that intrinsic histological abnormalities of the aortic wall present since infancy are an important causative factor leading to aortic root dilatation.

Methods and Results—We examined the aortic histology of 17 cases with TOF and PS/PA from our cardiac morphology archive and compared them with a control group of normal aortas. Measured circumference of the aortic root at the sinotubular junction and at the ascending aorta was indexed to the left ventricle. Aortic walls were studied by light microscopy with the use of various stains. Seventeen TOF cases (7 with PS, 10 with PA) including 7 infants, 2 children, and 8 adults were compared with 11 hearts with normal aorta. Aortic root circumference to left ventricular index and ascending aortic circumference to left ventricular index were 1.24 ± 0.25 and 1.37 ± 0.24, respectively, in the TOF group versus 0.89 ± 0.10 and 0.88 ± 0.11, respectively, in the control group (P < 0.001). Histological changes of grade 2 or 3 were present in 29% (medionecrosis), 82% (fibrosis), 35% (cystic medial necrosis), and 59% (elastic fragmentation) in the ascending aorta of the TOF group. Histology grading scores were significantly higher in the TOF group (median score, 7; range, 1 to 12) compared with normal controls (median score, 2; range, 0 to 6) and correlated with the ascending aortic circumference to left ventricular index (r = 0.525, P = 0.03).

Conclusions—There are marked histological abnormalities in the aortic root and ascending aortic wall of patients with TOF present from infancy, suggesting a causative mechanism for subsequent aortic root dilatation. (Circulation. 2005;112:961-968.)

Key Words: aorta ■ congenital heart defects ■ pathology ■ tetralogy of Fallot

Tetralogy of Fallot (TOF) is the most commonly encountered cyanotic congenital heart defect in infancy, with a frequency of nearly 10% of all congenital heart disease.1 The development and refinements of surgical repair techniques for TOF in the last 40 years have contributed to a steadily increasing population of adult survivors.2 Factors that may contribute to late morbidity in this cohort of patients and the need for further reintervention include residual right ventricular outflow tract obstruction, right ventricular dysfunction resulting from severe pulmonary regurgitation, tricuspid regurgitation from right ventricular dilatation, residual ventricular septal defect, development of atrial or ventricular tachyarrhythmias, and the often insidious development of progressive aortic root dilatation leading to aortic regurgitation and, with time, left ventricular dysfunction.

Although aortic root dilatation has been reported in patients with TOF3 even after reparative surgery,4 its underlying pathophysiology remains elusive. Progressive aortic root dilatation was thought to be due to increased aortic flow from right to left shunting in TOF patients before surgical repair.3,5 However, there has been some evidence of late progressive aortic root dilatation in a subset of TOF patients even after total corrective surgery. Niwa et al6 have shown that certain predisposing factors, including male sex, pulmonary atresia (PA), right aortic arch, and longer palliative-to-repair time, were associated with late aortic root dilatation. It was also postulated from this report that intrinsic properties of the aortic root itself may play an important part in aortic root dilatation, in addition to previous long-standing volume overload. Furthermore, abnormalities of smooth muscles, elastic fibers, collagen, and ground substance in the tunica media of the ascending aorta were found to be prevalent in the whole spectrum of congenital heart disease patients, predisposing to aortic dilatation, aneurysm, and/or aortic rupture.7

Received January 21, 2005; revision received April 13, 2005; accepted May 3, 2005.

From the Adult Congenital Heart Disease Unit (J.L.T., P.A.D., M.A.G.) and Department of Pediatrics (J.L.T., K.P.M., S.Y.H.), Royal Brompton Hospital and National Heart and Lung Institute, Imperial College, London, UK; and National Heart Center, Singapore General Hospital, Singapore (J.L.T.).

Correspondence to Dr S.Y. Ho, Cardiac Morphology, National Heart and Lung Institute, Imperial College London and Royal Brompton Hospital, Dovehouse St, London SW3 6LY, UK. E-mail yen.ho@imperial.ac.uk

© 2005 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org DOI: 10.1161/CIRCULATIONAHA.105.537928

961
TABLE 1. Histological Definitions and Grading of Tunica Media Sections

<table>
<thead>
<tr>
<th>Histology</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medionecrosis</td>
<td>Focal loss of smooth muscle cell nuclei in media</td>
<td>Focal loss consisting of &lt;1/3 of total width of media</td>
<td>Focal loss consisting of &gt;2/3 of total width of media</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Focal loss consisting of &lt;1/3 of total width of media</td>
<td>Focal loss consisting of 1/3 to 2/3 of total width of media</td>
<td>Focal loss consisting of &gt;2/3 of total width of media</td>
</tr>
<tr>
<td>Cystic medial necrosis (Mucoïd accumulation); “cysts” are observed in presence of intact elastic lamellae and fragmented elastic fibers</td>
<td>Minute foci within a single lamellar unit</td>
<td>Increased size and No. of “cysts” plus mucoid material &gt;1 lamellar unit</td>
<td>Large and extended “cysts” with fragmentation of elastic fibers</td>
</tr>
<tr>
<td>Elastic fragmentation</td>
<td>5 to 9 foci of elastic lamellae fragmentation in 1 microscopic field of ×200</td>
<td>5 to 9 foci of elastic lamellae fragmentation in 1 microscopic field of ×200</td>
<td>≥10 foci of elastic lamellae fragmentation in 1 microscopic field of ×200</td>
</tr>
</tbody>
</table>

We hypothesized that histological abnormalities of the ascending aorta would be present in TOF patients irrespective of age and would be related to aortic root dilatation.

**Methods**

We reviewed 17 postmortem heart specimens with TOF from our cardiac morphology archive of formalin-fixed specimens. All the postmortem specimens were obtained with consent. None were associated with atrioventricular septal defect. The degree of aortic root dilatation was inferred by direct measurement of the circumference along the internal surface of the sinotubular junction with the use of a thread. The ascending aorta was inspected, and if it appeared dilated, the inner circumference at the site of maximal dilatation of the ascending aorta and its distance from the sinotubular junction were also measured. Because the hearts were of different sizes, the measured aortic circumference at the sinotubular junction and the maximum ascending aortic dilatation was indexed to the size of the left ventricle, which was taken as the distance from the left atrioventricular junction to the endocardium of the left ventricular apex. The mitral valve was inspected to exclude any form of mitral inflow obstruction, which could potentially affect left ventricle size. Similar measurements of the aortic circumference and sections were taken from 11 normal heart specimens of varying sizes and ages for comparison. All direct measurements were performed at 3 separate occasions, and the average value was obtained.

Full-thickness samples of the tunica media, taken 5 mm distally from the sinotubular junction, were prepared for histology. Sections of 6-μm thickness were cut from paraffin blocks of each specimen. Four sister sections from each block were stained with hematoxylin and eosin, Masson’s trichrome, Alcian blue, and elastic van Gieson’s stain. These sections were examined under light microscopy for the presence of medionecrosis, fibrosis, cystic medial necrosis, and elastic fragmentation. When lesions were absent, they were denoted as 0, and when they were present, they were graded 1 to 3 according to the criteria adapted from Schlattmann and Becker4 and from de Sa et al9 (Table 1). One observer made the examinations through the full thickness of the media on 3 separate occasions.

Additional random sections were taken from the aortic sinuses (aortic root) and descending thoracic aortas of adult specimens. In 6 of the 11 normal hearts (2 infants, 1 child, 3 adults), further sections of the ascending aorta, 5 mm above the sinotubular junction, were analyzed histologically for the presence of medionecrosis, fibrosis, cystic medial necrosis, and elastic fragmentation changes.

**Statistical Analysis**

Data analysis was performed with the use of SPSS for Windows (version 10.0, SPSS). Continuous data are expressed as mean with SD or median with range when appropriate. Nominal data are expressed as numbers and percentages. Group means were compared by the Mann-Whitney test. Categorical variables were analyzed by χ² tests. A 2-tailed probability value of <0.05 was considered significant.

**Results**

Seventeen TOF cases (7 with pulmonary stenosis [PS], 10 with PA) including 7 infants (3 male; median age, 8 months; range, 3 days to 12 months), 2 children (both female; aged 3 and 6 years), and 8 adults (5 male; median age, 42 years; range, 20 to 57 years) were compared with 11 control cases (5

**TABLE 2. Clinical Features of TOF Group Divided Into Respective Age Groups**

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Male</th>
<th>Age Deceased</th>
<th>PA</th>
<th>Right Aortic Arch</th>
<th>Palliative Surgery Only (Number, Age at Palliation, and Type of Surgery)</th>
<th>Both Palliative and Corrective Surgery (Number, Age at Palliation [P] and Correction [C], and Type of Surgery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants (n=7)</td>
<td>3</td>
<td>Median age, 8 mo (range, 3 d to 12 mo)</td>
<td>3</td>
<td>2</td>
<td>n=1; age 3 d; BT shunt</td>
<td>0</td>
</tr>
<tr>
<td>Children (n=2)</td>
<td>0</td>
<td>3 and 6 y</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adults (n=8)</td>
<td>5</td>
<td>Median age, 42 y (range, 20–57 y)</td>
<td>6</td>
<td>5</td>
<td>n=3; age 7, 39, 45 y; 2 BT shunts, 1 Waterston shunt</td>
<td>n=2; age 10 y (P), 15 y (C), TOF/PS repair; age 13 y (P), 25 y (C), TOF/PS repair</td>
</tr>
</tbody>
</table>

BT indicates Blalock-Taussig.
adults, 3 children, 3 infants) with normal hearts and normal aortas (Table 2). The TOF patients died between 1965 and 1999, and the controls died between 1970 and 1995. Clinical data on the TOF necropsy specimens were limited. PA was present in 59% of TOF cases (3 infants, 1 child, 6 adults), and right aortic arch was noted in 41% of the TOF cases (2 infants and 5 adults). The majority of the TOF patients (65%) had no prior palliative or corrective surgery. Four TOF patients (1 infant, 3 adults) had palliative surgery only, and 2 adult TOF patients had palliative surgery followed by total corrective surgery a few years later.

### Aortic Size

The measured aortic circumference indexed to left ventricular size was significantly greater in the TOF group (1.24±0.25) than in the normal group (0.89±0.10) \((P<0.001)\) (Figure 1a). Within the TOF group, there was no significant difference between the aortic root circumference to left ventricular index of the 10 patients with PA and 7 patients with PS (mean, 1.32±0.24 versus 1.13±0.24; \(P=0.11\)) (Figure 1b). Twelve of the 17 TOF specimens and 10 of 11 normal hearts had sufficient ascending aorta present for measurement of the widest ascending aortic circumference observed 5 to 10 mm beyond the sinotubular junction. Maximum ascending aortic circumference indexed to left ventricular size was significantly greater in the TOF group (1.37±0.24) than in the normal group (0.88±0.11) \((P<0.001)\) (Figure 1c). Again, there was no significant difference in the ascending aortic circumference to left ventricular index between the PA and PS subgroups (mean, 1.40±0.26 versus 1.32±0.23; \(P=0.89\)) (Figure 1d).

### Histology

#### Medionecrosis

In the TOF group, medionecrosis of grade \(\geq 2\) was present in 29% (5 of the 17 necropsy specimens) of the ascending aorta, including 2 infants. In the adult TOF group in which additional tissue was removed, medionecrosis of grade \(\geq 2\) was present in at least 1 of the aortic sinuses in 88% (7 of the 8 adults) but none in the descending thoracic aorta. In the

---

**Figure 1.** a, Aortic root circumference to left ventricular (LV) index \((A_{oi})\) in TOF group \((n=17)\) and normal group \((n=11)\). b, Aortic root circumference to left ventricular index \((A_{oi})\) in TOF group with PA \((n=10)\) and PS \((n=7)\). c, Maximum ascending aortic circumference to left ventricular index \((ascA_{oi})\) in TOF group \((n=12)\) and normal group \((n=10)\). d, Maximum ascending aortic circumference to left ventricular index \((ascA_{oi})\) in TOF group with PA \((n=7)\) and PS \((n=5)\).
selected normal group, there were no medionecrosis changes of grade 2 or 3 and only grade 1 changes in 2 of the 3 adult specimens and none in the rest.

**Fibrosis**

Fibrosis of grade ≥2 was present in 82% (14 of the 17) of the TOF group, including 6 infants and 1 child. In the adult TOF group, fibrosis of similar grade was also present in at least 1 of the aortic sinuses in all 8 patients and in descending thoracic aorta of 2 patients. Once again, there were no grade 2 changes in the normal controls, and only minor grade 1 fibrosis was present in 2 of the 3 selected adult normals and in none in the remaining child and infant specimens.

**Cystic Medial Necrosis**

Cystic medial necrosis of grade ≥2 was present in the ascending aorta in 35% (6 of 17) of the TOF group, including 2 infants and 1 child. Once again, it was also found in at least 1 of the aortic sinuses in 38% of the TOF adults (3 of the 8 adults) and in the descending aorta in 25% of the TOF adults (2 of the 8 adults). In the control group, cystic medial necrosis of grade 2 was found in only 33% (1 of the 3 adults) and in none in the children and infant group. Minor grade 1 changes were present in 2 of the normal adult controls and in 1 normal child.

**Elastic Fragmentation**

Elastic fragmentation of grade ≥2 was present in 65% (11 of 17) of the TOF group, including 5 infants and 1 child. In the adult TOF group, similar findings were present in 1 of the aortic sinuses in 88% (7 of the 8 adults) and in the descending aorta in 25% (2 of the adults). In the selected normal group, elastic fragmentation of grade 2 was present in 1 of the normal controls and in none in the infant/children group. Minor grade 1 changes were present in 2 of the 3 normal adults and in 1 child.

**Elastic Lamellae**

In the TOF group, elastic lamination was noted to be disrupted in 3 of the 7 infants (43%), and hence lamellae count was not possible. In the remaining 4 specimens, the elastic lamellae present ranged from 48 to 93 U. In the 2 TOF children, the number of elastic lamellae was 69 in both children. In the adult TOF groups, 50% (4 of the 8 adults) had disrupted or disarrayed elastic lamination, and the remaining 4 had normal appearance of elastic lamellae, ranging from 52 to 67 U. In the normal group, the elastic lamellae present ranged from 49 to 66 U across the 3 age groups.

Summaries of the histological findings are shown in Figures 2 to 4. In addition, there were no significant differences between male sex, PA, right aortic arch, and previous palliative surgery in relation to the development of grade 2 or 3 histological changes. The degree of aortic root dilatation as measured by ascending aortic circumference to left ventricular index did not correlate significantly with these histological changes.

**Histology Grades Score**

To further evaluate the relationship between the severity of the histological changes to the aortic root circumference to left ventricular index and ascending aortic circumference to left ventricular index, a grading system was devised to grade the severity of the histological changes, as shown in Table 3. There was a significant difference between the median histological grading score for the TOF group (median score = 7; range, 1 to 12) versus the normal control group (median score = 2; range, 0 to 6) (P = 0.01). The histology scores correlated positively with the maximum ascending aortic circumference to left ventricular index (r = 0.525, P = 0.03), as shown in Figure 5, but not with the aortic root circumference to left ventricular index (r = 0.353, P = 0.10). The tissue specimen removed for histological analysis was taken at the level of the ascending aorta, 5 mm beyond the sinotubular junction; because the maximum ascending aortic dilatation also occurred between 5 to 10 mm beyond the sinotubular junction, it was not surprising that there was better correlation of the histology scores with ascending aortic circumference to left ventricular index than with aortic root circumference to left ventricular index, which is exactly at the level of the sinotubular junction.
Discussion

This study shows that significant aortic root dilatation in TOF patients with PS or PA is present from early infancy, and this relates to intrinsic histological abnormalities of the aortic wall.

Aortic Dilatation

Aortic circumference from the necropsied heart specimens in our study showed significant dilatation in the TOF group across all age groups compared with normal hearts.

Other researchers and ourselves3–6,10–12 have reported aortic root dilatation potentially leading to aortic regurgitation in TOF patients with PS or PA. The aortic root dilatation is often progressive, leading to aortic valve replacement, and can occur in unoperated patients3,5,11 as well as in TOF patients late after repair.4,6,10,12 Niwa et al6 reported aortic root dilatation in 15% of adult patients after TOF repair. These patients had longer shunt-to-repair interval as well as higher prevalence of PA and right aortic arch. In our data, 59% and 41% of the TOF group had PA or right aortic arch, respectively. As shown, the majority of our patients (65%) were unrepaired or unoperated, including 6 infants and 2 children. We are not aware of any published series of aortic root dilatation in necropsied specimens from such a young cohort.

Aortic root dilatation in unoperated TOF patients has been attributed to the increased blood flow from both ventricles to the overriding aorta.3,5,13 This was further supported by the observation that unoperated TOF patients with PA rather than PS had a higher incidence and greater aortic root dilatation from the presumptive higher aortic flow.3,5,13 In operated TOF patients, the cause of aortic root dilatation is thought to be predominantly secondary to chronic hemodynamic stress from volume overloading of the aorta before reparative surgery.4,10,14 The presence of significant aortic root dilatation in early infancy in our TOF group strongly suggests that intrinsic aortic wall abnormalities reported here also play a causative role in aortic root dilatation, in addition to the effects of chronic volume overloading and physiological changes associated with aging.15

Histological Abnormalities

Niwa et al7 had shown that there were abnormal histological changes in intraoperative biopsies and necropsy specimens of the great arteries taken from a spectrum of congenital heart disease lesions (Table 4). Necropsy specimens from the ascending aorta of 15 adult patients with TOF showed grade 2 and 3 changes in the aortic media on light and electron microscopy. Our study confirms these early findings. Further-
more, our study extends the histological examination to infants and children with TOF with direct comparison to control specimens with normal aortas.

The normal aortic tunica media consists of layers of longitudinally arranged elastic lamellae interspersed with smooth muscle cells and collagen fibrils in a mucopolysaccharide ground substance. The number of elastic lamellae is greatest in the ascending aorta (Table 4) and decreases in number across the descending thoracic aorta. With increasing age, there is a corresponding decrease and degeneration of the elastic lamellae and partial replacement with collagen, resulting eventually in histological changes such as medionecrosis, fibrosis, cystic medial necrosis, and elastic fragmentation. Schlatmann et al (Table 4) had shown that in the normal aortic wall of patients aged >20 years (youngest aged 18 months), there were no histological changes such as fibrosis and elastic fragmentation of grade ≥2, and similar grade 2 changes of medionecrosis and grade 3 changes of cystic medial necrosis were rare. Histological findings from a selection of our normal hearts across the 3 age groups were consistent with these findings and showed that the histological changes of medionecrosis, fibrosis, cystic medial necrosis, and elastic fragmentation (up to grade 2) were present in some normal adults but were completely absent in infants. Furthermore, more marked histological changes observed with increasing age within the TOF group (median score in infant/children of 4 versus median score in adults of 7) suggest that aging coupled with aortic volume overloading on top of the intrinsic aortic wall abnormalities described has an additional adverse effect on the aortic histology and thus aortic dilatation.

Our study showed that histological changes such as fibrosis and elastic fragmentation of grade ≥2 with disrupted or disarrayed elastic lamination were frequently seen not only in the ascending aorta of adult TOF patients but also in infants and neonates, some only a few days old. Fibrosis, elastic fragmentation, and disruption of elastic lamellae seemed to occur in greater proportion among TOF patients across all age

### TABLE 3. Histology Grading Score for TOF vs Normal Controls*

<table>
<thead>
<tr>
<th>Heart Specimens</th>
<th>Medionecrosis Grades</th>
<th>Fibrosis Grades</th>
<th>Cystic Medial Necrosis Grades</th>
<th>Elastic Fragmentation Grades</th>
<th>Elastic Lamellae Units</th>
<th>Histology Grading Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOF†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant 1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>93</td>
<td>3</td>
</tr>
<tr>
<td>Infant 2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>49</td>
<td>2</td>
</tr>
<tr>
<td>Infant 3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>72</td>
<td>4</td>
</tr>
<tr>
<td>Infant 4</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>69</td>
<td>7</td>
</tr>
<tr>
<td>Infant 5</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>Disrupted</td>
<td>8</td>
</tr>
<tr>
<td>Infant 6</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>Disrupted</td>
<td>9</td>
</tr>
<tr>
<td>Infant 7</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>Disrupted</td>
<td>12</td>
</tr>
<tr>
<td>Child 1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td>Child 2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>69</td>
<td>1</td>
</tr>
<tr>
<td>Adult 1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>Disrupted</td>
<td>7</td>
</tr>
<tr>
<td>Adult 2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>Disrupted</td>
<td>10</td>
</tr>
<tr>
<td>Adult 3</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>Disrupted</td>
<td>9</td>
</tr>
<tr>
<td>Adult 4</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>52</td>
<td>6</td>
</tr>
<tr>
<td>Adult 5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>57</td>
<td>4</td>
</tr>
<tr>
<td>Adult 6</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>65</td>
<td>5</td>
</tr>
<tr>
<td>Adult 7</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>67</td>
<td>7</td>
</tr>
<tr>
<td>Adult 8</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>Disrupted</td>
<td>10</td>
</tr>
<tr>
<td>Normal‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>Infant 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td>Child 1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td>Adult 1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>66</td>
<td>4</td>
</tr>
<tr>
<td>Adult 2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>58</td>
<td>6</td>
</tr>
<tr>
<td>Adult 3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>63</td>
<td>2</td>
</tr>
</tbody>
</table>

*Points allocated for various grades were as follows: grade 0 = 0 points; grade 1 = 1 point; grade 2 = 2 points; grade 3 = 3 points; elastic lamellae units with no disruption = 0 points; if units were disrupted = 2 points.
†TOF group histology grading median score = 7 (range, 1–12); TOF infant and children subgroup median score = 4 (range, 1–12); TOF adult subgroup median score = 10 (range, 4–10).
‡Normal control histology grading median score = 2 (range, 0–6); normal infant and children subgroup median score = 0 (range, 0–2); normal adult subgroup median score = 4 (range, 2–6).
groups in comparison to histological changes like medionecrosis and cystic medial necrosis. Cystic medial necrosis is most marked in Marfan syndrome, in which the genetic defect in fibrillin-1 results in increased elastin degradation by metalloproteinases (Table 4). Medial smooth muscle cell apoptosis leading to cystic medial necrosis was thought to be the mechanism for ascending aortopathy in bicuspid aortic valve disease. In adults with abdominal aortic aneurysm, 1 of the hallmark histological feature is the extensive loss of elastin in the media and adventitia, which contributes to the initiation and expansion of the aortic aneurysm. The loss of elasticity in the aortic root and ascending aorta is much earlier in life. Elastic fragmentation and disruption occurred earlier and more frequently in our TOF patients in comparison to either Marfan patients or patients with bicuspid aortic valve, in which the predominant histological abnormality is cystic medial necrosis. Elastin has a long half-life of 40 to 70 years, and the loss of elastin in adults is most likely a manifestation of elastolysis rather than insufficient synthesis. In children and particularly in infants with TOF and PA/PS, whether the loss of elastin is due to increased elastolysis stimulated by the shear and increased flow volume through the aorta or due to intrinsic problems of elastin synthesis remains uncertain. Could this intrinsic abnormality be an expression of a hitherto unrecognized genetic defect involving the cellular function (eg, metalloproteinases) of the aortic media in TOF patients, or is this a programmed apoptosis? If apoptosis of smooth muscle cell (Table 4) is the underlying mechanism for bicuspid aortopathy, could the elastin fragmentation and disruption seen in our TOF patients be the final expression of a programmed or triggered apoptosis of elastic fibers? The obvious triggering factor here would be the shearing force from volume overloading of the aorta early in life, with the greatest stress being generated at the aortic root and ascending aorta. This hypothesis is supported by our analysis of the aortic histology from the aortic sinuses and descending thoracic aorta in the adult TOF group, suggesting an even greater degree of histological changes in the aortic root at the aortic sinuses than at the ascending aorta, with near normalization of histology in the descending thoracic aorta.

**Limitations of the Study**

This study is limited by the number of heart specimens available from our morphological archive, which is also used for education. In necropsied hearts, formalin fixation causes shrinkage of valvar and aortic tissue, and hence direct comparison with available normograms of indexed aortic root dimensions cannot be made. Tissue samples from the aortic sinuses of infants and children with TOF were not removed for further histological analysis because this could cause further destruction to the small heart specimens, which are also needed for teaching and education. Furthermore, the observer making the measurements of the aortic dimensions

---

**TABLE 4. Comparison of Previous Published Studies on Aortic Histology of Specific Conditions**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Author/Journal</th>
<th>Population</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 Types of congenital heart disease</td>
<td>Niwa et al, Circulation, 2001</td>
<td>102 patients (86 intraoperative biopals, 16 necropsy specimens of ascending aorta; mean age 32 ±6 y</td>
<td>Aortic medial abnormalities of smooth muscle, elastic fibers, collagen, and ground substance were prevalent in a variety of congenital heart diseases</td>
</tr>
<tr>
<td>Aging aorta</td>
<td>Schlatmann et al, Am J Cardiol, 1977</td>
<td>100 normal aortas across entire age range</td>
<td>Histological changes in medial area of aorta correlated with age</td>
</tr>
<tr>
<td>Bicuspid aortic valve</td>
<td>Bondeman et al, Circulation, 1999</td>
<td>Ascending aortic wall intraoperative specimens from 32 patients (16 bicuspid aortic valve with/without aortic dilatation)</td>
<td>Apoptosis is mechanism underlying smooth muscle cell loss in ascending aortas of bicuspid aortic valve carriers</td>
</tr>
<tr>
<td>Marfan syndrome</td>
<td>Segura et al, Circulation, 1998</td>
<td>Thoracic aortic aneurysm of 7 patients with Marfan syndrome</td>
<td>All thoracic aortic aneurysms showed cystic medial necrosis with loss of elastic fibers and smooth muscle cells; elastin in Marfan syndrome was more easily degraded by matrix metalloproteinases than normal elastin</td>
</tr>
<tr>
<td>Abdominal aortic aneurysm</td>
<td>Brophy et al, Ann Vasc Surg, 1991</td>
<td>Comparison of normal and diseased aorta in humans</td>
<td>Major tissue inhibitor of metalloproteinases is diminished in abdominal aortic aneurysm, leading to increased proteolysis and elastin loss</td>
</tr>
</tbody>
</table>
could not be blinded to the pathology, normality, or size or to whether there was previous surgical repair because we needed to retain the aortic roots on the specimens.

The results from this study did not take into consideration any coexisting heritable disorders of connective tissue or metabolic-, drug-, or toxin-induced syndromes that may contribute to the aortopathic process because of unavailable patient records and clinical data in the TOF group. Clinical and echocardiographic data on the necropsied hearts from the normal group were incomplete and hence not included. Larger-scale clinical studies of the aorta commencing in infancy are required and may shed further light on risk stratification for aortic dilatation and the rate of its progression.

Conclusions
There are intrinsic histological changes ranging from medioclasis, fibrosis, cystic medial necrosis, and elastic fragmentation to elastic lamellae disruption in TOF patients with either PS or PA, which are present as early as a few days after birth. These abnormalities seem to contribute to subsequent progressive aortic dilatation, and this warrants further investigation.

Acknowledgments
Dr Tan was supported by grants from the Health Manpower Development Plan (Singhealth, Ministry of Health, Singapore) and the National Heart Center, Singapore. Dr Davlouros was supported by the Greek Cardiac Society and the Royal Brompton Hospital Clinical Research Committee. Dr Gatzoulis and the Royal Brompton Adult Congenital Heart Program were supported by the British Heart Foundation. Dr Ho was funded by the Royal Brompton and Harefield NHS Trust Charitable Fund. We thank Dr Roberto Zecchel from Treviso, Italy, for help in the initial data collection.

References
Intrinsic Histological Abnormalities of Aortic Root and Ascending Aorta in Tetralogy of Fallot: Evidence of Causative Mechanism for Aortic Dilatation and Aortopathy

J.L. Tan, P.A. Davlouros, K.P. McCarthy, M.A. Gatzoulis and S.Y. Ho

_Circulation_. 2005;112:961-968; originally published online August 8, 2005;
doi: 10.1161/CIRCULATIONAHA.105.537928

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/112/7/961

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/