Analysis of the aortic root in patients with tetralogy of Fallot undergoing early repair: Form follows function

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Objective: Aortic root dilatation remains of concern late after repair for tetralogy of Fallot (ToF). We have previously demonstrated that the aortic root size decreases progressively after early correction. To test whether the aortic wall changes are intrinsic or acquired, a histologic study was performed of infants at early repair.

Methods: From 24 of 31 infants undergoing ToF repair (mean age, 5.9 ± 4.9 months), ascending aortic biopsies were obtained. After conventional staining, the specimens were given a histologic score (0-3) for medionecrosis, ground substance accumulation, fibrosis, smooth muscle cell disarray, and apoptosis. The aortic root diameters were monitored using echocardiography for ≤ 2 years postoperatively.

Results: Nearly one half of the specimens showed signs of fibrosis (45%), with increased mucoid accumulation in 15% and elastin fragmentation in 5%. The aortic root was dilated in all infants at operation (mean indexed diameter—annulus, 38.1 ± 5.8 ; sinus, 47.8 ± 6 ; sinotubular junction, 38.7 ± 5.4 mm/m²) and had regressed significantly within 2 years (mean indexed diameter—annulus, 27.9 ± 4.7 ; sinus, 36.4 ± 5.1 ; sinotubular junction, 30.4 ± 6 mm/m²; $P < .0001$). The total histology score showed no correlation with indexed diameter regression at 2 years.

Conclusions: Infants with ToF undergoing repair around 6 months old already had limited features of aortic wall degradation at surgery. Echocardiographic follow-up, however, demonstrated significant reductions in the indexed aortic diameters. We presume that early repair of ToF will prevent a disproportionate aortic diameter increase by halting progression of the limited histologic changes potentially predisposing to late aortic dilatation. (J Thorac Cardiovasc Surg 2014;148:1555-9)

Aortic root dilatation in patients with tetralogy of Fallot (ToF) remains an issue in adolescent and adult patients late after repair. The underlying mechanism is unclear; both hemodynamic¹ and intrinsic wall abnormalities²⁻⁴ have been suggested. Dilatation of the ascending aorta in ToF has been related to long-standing aortic volume overload in the context of right outflow obstruction and a right-to-left shunt.⁵ A right-sided aortic arch, male gender, aortopulmonary shunts, and complete repair at an older age have been associated with late aortic dilatation. In a previous retrospective observational study, we demonstrated that the indexed aortic root diameters decrease progressively when correction has been performed early in life and that the originally dilated aortic root in ToF normalizes in size at the level of the annulus and sinotubular junction

(STJ) within 7 years after early repair in most patients, although with less reduction at the sinuses of Valsalva.⁶ To test whether the aortic wall changes in ToF are intrinsic or acquired, we performed a prospective histologic study, focusing on the ascending aortic wall characteristics in infants at early repair. Using longitudinal follow-up of the aortic root dimensions in the repaired patients, we aimed to discover a relationship between the histologic features and the evolution of the originally dilated aortic root and to define the risk factors for persistent aortic dilatation.

METHODS

Patient Population

A prospective design was used for the present study. A total of 31 consecutive patients who had been scheduled for complete repair of ToF from September 2008 to November 2010 at the Cardiac Centre of the University Hospital Ghent were eligible for the present study. Patients with pulmonary atresia or a ventricular septal defect were excluded. For 24 patients, informed consent was obtained for removal of a segment of the ascending aortic wall during ToF correction for histologic examination. The mean age, weight, and height at operation was 5.9 ± 4.9 months, 6.3 ± 1.8 kg, and 64 ± 7 cm, respectively. The male-to-female ratio was 1.6. Four patients (13%) had previously received a neonatal Blalock-Taussig shunt at a mean age of 7 days after birth because of duct dependent pulmonary circulation or severe cyanotic spells. The patients who had undergone shunt placement underwent complete repair at a mean age of 4.6 ± 1.2 months. A right aortic arch was present in 4 patients (13%). In 2 patients, a genetic syndrome was identified (22q11 duplication, 4q26 duplication). All patients were prospectively seen in the outpatient clinic at 6 months, 1 year, and 2 years postoperatively for physical and

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Disclosures: Daniel De Wolf reports consulting fees from Actelion and lecture fees from Nutricia. All other authors have nothing to disclose with regard to commercial support.

Received for publication Aug 10, 2013; revisions received Nov 22, 2013; accepted for publication Nov 29, 2013; available ahead of print Feb 4, 2014.

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0022-5223/\$36.00

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<http://dx.doi.org/10.1016/j.jtcvs.2013.11.044>

Abbreviations and Acronyms

BSA = body surface area
STJ = sinotubular junction
ToF = tetralogy of Fallot

echocardiographic examination. The hospital ethics committee approved the study (Belgian registration no. B67020084368).

Methods

The operative technique for ToF repair for all patients was a standard transatrial–transpulmonary approach, with closure of the ventricular septal defect using a Gore-Tex patch (W.L. Gore & Associates, Inc, Newark, Del), and infundibular resection through a right atriotomy, supplemented with a limited transannular autologous pericardial patch, if necessary. A full-thickness fragment of about 7×2 mm of the anterior wall of the ascending aorta, 4 to 5 mm above the STJ, was removed at the site of the small fat pad where the cardioplegia needle was inserted. The biopsy was taken after aortic crossclamping, immediately after delivery of antegrade crystalloid cardioplegia solution, and the aortic incision was closed with a running monofilament stitch. The patient and operative details are listed in Table 1.

Each aortic wall specimen was fixed immediately in 4% buffered formalin solution for a minimum of 4 hours at room temperature, and thin 3- μ m sections were taken. The slides were stained with hematoxylin and eosin, and different special histochemical Masson's trichrome, elastica von Gieson's, and Alcian blue staining. The sections were examined with a light microscope (Olympus BX45, magnification 4–400 $\times$) and evaluated by 1 pathologist (D.C.) for smooth muscle disarray, focal fragmentation of elastic lamellae, the amount of ground substance, cystic medionecrosis, degree of fibrosis, and apoptosis. Every component of the tunica media was graded from 0 to 3, with grade 0 indicating no disturbance and grade 3, the most severe form of histologic changes, using previously described histologic definitions.^{3,7} A total histology score for each patient was calculated, taking into account a possible maximum score of 3 for the tunica media components (medionecrosis, fibrosis, accumulated ground substance, and elastic fragmentation). Medionecrosis was defined as a focal loss of smooth muscle cell nuclei in the media. Fibrosis was defined as an increase in interstitial collagen. The accumulation of ground substance was characterized by a noninflammatory loss of smooth muscle cells and replacement with mucoid material. Elastic fragmentation was defined as focal fragmentation of elastic lamellae in the aortic media.⁴

At surgery and each scheduled postoperative visit, the anthropometric data were recorded, and the body surface area (BSA) was calculated according to the Mosteller formula for children. Transthoracic echocardiography was performed, with systematic recordings of the aortic root dimensions. Measurements of the aortic root were performed from the parasternal long axis, at mid-systole, perpendicular to the long axis of the aorta, focusing on the internal diameter of the aortic annulus, the sinus of Valsalva and STJ. The absolute diameters were recorded in millimeters and are expressed as z-values using standard algorithms.⁸ The z-value represents the number of standard deviations from the mean diameter at each level, normalized for the patient's BSA, gender, and age. In addition, the obtained root diameters indexed to the BSA (in mm/m²) and to the square root of the BSA (in mm/m) were calculated for each patient, because these parameters have the potential to eliminate the differences between boys and girls and do not take age into account.^{9,10}

Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences, version 19.0, software (SPSS Inc, Chicago, Ill). The descriptive data are presented as the mean $\pm$ standard deviation. To compare the

z-values and the indexed root diameters of the aortic annulus, aortic sinus, and STJ preoperatively and at 6 months, 1 year, and 2 years postoperatively, with the possible influence of the histology score as a covariate, analysis of variance with repeated measures was used. Bivariate analysis was used to determine the correlation of histology score with the indexed root diameters at surgery and with the decrease in indexed root diameters after 2 years. To determine the influence of categorical variables (ie, previous shunt, right aortic arch, gender) on aortic root size, an independent samples *t* test was used. Two-sided *P* values were computed.

RESULTS

The postoperative course was uneventful for all 31 patients. No complications from the aortic biopsy or revisions for bleeding were noted. All 31 patients were followed up for a minimum of 2 years postoperatively.

Histopathologic Findings for Ascending Aorta

None of the 24 aortic specimens showed any histologic signs of smooth muscle cell necrosis or smooth muscle disarray. The distribution of the other histologic changes in the tunica media is depicted in Figure 1. In 45% of the patients, fibrosis grade 2 or more was found, with a mean histologic score of 1.4 (range, 0–3) for the group. An increased accumulation of mucoid ground substance was encountered in 15% of biopsy specimens (mean histologic score, 0.6 for the group; range, 0–2). Second-degree fragmentation of the elastic fibers was spotted in only one 7-month-old patient with a right aortic arch and the largest annulus z-score of the series (16.7 mm; z-score, 8.3). No apoptosis was found; however, the preapoptotic features were not studied. The total histology score for each patient was calculated, with a mean histology score of 2.5 (range, 1–6).

Evolution of Aortic Root Diameter

The mean z-values of the aortic annulus, sinus of Valsalva, and STJ were abnormal at all points, with, however, large standard deviations. These decreased progressively over time to 2 years postoperatively. The changes in z-score at the level of the aortic annulus were significant between the operation and 1 and 2 years postoperatively. The z-score changes at the sinus and STJ level over time were not significant (Figure 2).

The change in the indexed aortic diameters of the aortic annulus, sinus of Valsalva, and STJ was very significant between each interval—apart from the STJ diameter from the operation to the 6-month follow-up point, with a continuous, but steady, indexed diameter decrease from surgery to ≤ 2 years later. The original histology score had no influence on the degree of the size reduction at any of the aortic root levels (Wilks' λ *P* = .7). The indexed aortic diameter resulted in a more consistent measure to describe the gradual reduction of the root diameters compared with the z-values (Figure 3).

TABLE 1. Patient and operative characteristics (n = 31)

Characteristic	Value
Gender	
Male	19
Female	12
Right aortic arch (n)	4 (13)
Blalock-Taussig shunt (n)	4 (13)
Age at correction (mo)	5.9 ± 4.9
Weight at correction (kg)	6.3 ± 1.8
Height at correction (cm)	63.8 ± 6.8
Body surface area at correction (m ²)	0.33 ± 0.06
Extracorporeal circulation duration (min)	94 ± 27
Crossclamp time (min)	55 ± 17

Data presented as n (%) or mean ± standard deviation.

No relationship was found between the total histology score and the aortic root diameters at surgery nor with the degree of the indexed diameter regression of the aortic root after 2 years.

Relationship of Aortic Diameters With Arch Side, Gender, or Previous Shunt

A right aortic arch has been described previously as a risk factor for persistent aortic dilatation after ToF repair. We did not find larger aortic root diameters at surgery in the patients with a right arch. The indexed aortic root diameter in the patients with a right arch also decreased over time but remained significantly larger at 2 years at the sinus of Valsalva level ($P = .042$) and at 6 months and 2 years at the STJ level ($P = .01$).

In female patients only, the indexed annulus diameter at surgery was larger than in the male patients (female, 41.1 ± 5 vs male, 36.2 ± 5.4 mm/m²; $P = .011$). Of the female patients, 75% needed a transannular patch compared with 53% of the male patients, reflecting a larger discrepancy

between the aortic and pulmonary valve annulus in the female group. During follow-up, however, no gender differences persisted at any level.

In the patients who had received a shunt before complete repair, the indexed annulus and STJ diameter were significantly larger only at surgery (no shunt, annulus, 37.5 ± 5.8 mm/m² vs shunt, annulus, 42.02 ± 3.1 mm/m², $P = .05$; no shunt, sinus, 38.1 ± 5.3 mm/m² vs shunt, sinus, 44.1 ± 2.7 mm/m², $P = .034$). During follow-up, however, the size difference between the shunted and nonshunted group was obliterated. The age at complete correction was also comparable for the shunted and nonshunted patients (6.1 ± 2.2 vs 4.6 ± 1.3 months; $P = .75$; Table 2).

DISCUSSION

A size discrepancy between a large ascending aorta and a smaller pulmonary root is a typical feature of uncorrected TOF. In a previous retrospective study, we demonstrated a significant regression of the indexed aortic root diameters over time after early repair in infancy, with a chance of normalizing the initially dilated root within 7 to 8 years.⁶

In the present study, we investigated whether histologic abnormalities were already present in the aorta of young infants with TOF at early repair and, whether a correlation could be found between the histology score and the evolution of the aortic root diameter after repair.

In our department, a policy of elective transatrial–transpulmonary correction around the age of 6 months has been adopted consistently in previous years and has resulted in a very homogeneous patient population. The few patients who had received a neonatal shunt also underwent definitive repair around the age of 6 months.

The aortic biopsy was taken right above the aortic valve commissures, where the aorta is usually dilated. In this

FIGURE 1. Degree of histologic disturbance found in aortic biopsy specimens.

FIGURE 2. Evolution of aortic root z-scores. *STJ*, Sinotubular junction.

aspect, our approach differed from that used in the study by Chowdhury and colleagues,⁴ in which the biopsy was removed from the site of aortic cannulation, where the ascending aorta has already tapered in size. The proximal ascending aorta a few millimeters above the STJ forms a continuity with the dilated root and was defined as the biopsy site closest to the root, without compromising or distorting the geometry of the aortic cusps.

Even at this young age, in more than one half of the specimens, some degree of histologic disturbance was found, mainly increased fibrosis and accumulation of ground substance. More severe forms of vessel wall degradation were not encountered, except for grade 2 elastin fragmentation in a 7-month-old girl with a right aortic arch and a very enlarged aorta. When considering the total histology score of the aorta, however, we could not define a correlation with the original indexed root diameters nor with the decrease in indexed root diameters after 2 years postoperatively. In the only other published prospective histologic

FIGURE 3. Evolution of indexed aortic root diameters over time. *Anl*, Indexed aortic annulus diameter; *Sinl*, indexed aortic sinus diameter; *STJl*, indexed sinotubular junction diameter; *OP*, operation. * $P < .05$ for change at each aortic root component; § $P < .05$ for change at level of annulus and sinus.

TABLE 2. Analysis of patient risk factors and aortic root diameters (univariate analysis)

Factor	P value
Right aortic arch	
Indexed sinus diameter at 2 y	.04
Indexed STJ diameter	
At 6 mo	.01
At 2 y	.01
Female gender	
Indexed annulus diameter at surgery	.01
Previous shunt	
Indexed annulus diameter	
At surgery	.05
Indexed STJ diameter	
At surgery	.03

STJ, Sinotubular junction.

study on the aorta in ToF,⁴ a much greater prevalence and more severe forms of vessel wall disturbance were found. This can probably be explained by the older age at operation (mean, 9 years) in that study, suggesting a relationship between the duration of volume overload and cyanosis on the one hand and increasingly worse histology scores of the aortic wall on the other. Autopsy studies on normal aortas have shown that fibrosis, cystic medionecrosis, and elastin fragmentation are virtually absent in the first decade of life and increase with age, suggesting that the processes of injury and repair are initiated by a continuous hemodynamic impact throughout life.^{11,12} We speculate that increased fibrosis might be the first feature of a medial reparative process, and that, once medionecrosis, smooth muscle cell disarray, and severe elastin fragmentation have occurred, repair mechanisms might not be able to completely restore the aortic wall integrity after ToF repair. This could explain why an increased stiffness and greater pulse wave velocity in the aorta of patients with repaired ToF have been described, several years after the correction.¹³⁻¹⁵ Although the measurements in these studies were only snapshots that could not take into account the evolution of the vessel wall characteristics over time, they support the idea that when the repair is performed at a later age, irreversible damage to the aorta has already occurred. In patients with Marfan syndrome, it has also been demonstrated that loss or severe fragmentation of the elastic fibers leads to increased aortic stiffness, preceding aortic dilatation.¹⁶ It remains unclear why significant histologic abnormalities could only be demonstrated in one half of our patients. In the present study, we did not consider the presence of genetic variations, such as matrix metalloproteinase-9 or fibrillin-1 polymorphisms, known to modulate aortic stiffness.^{17,18}

Irrespective of the total histology score at surgery, we found a significant and consistent decrease in the indexed aortic root diameters during the follow-up period, ≤ 2 years postoperatively. This finding supports the results from our

previous study⁶ and has proved that, in a homogenous cohort of patients with ToF repaired early in infancy, the relative dilatation of the ascending aorta actually decreases with the growth of the patient, once a normal hemodynamic status has been restored at a young age. Thus, we would like to suggest the usefulness of using the aortic diameters indexed to body surface area, rather than reporting the z-scores, when describing large vessel diameters in young children, because the former parameter is less dependent on gender or age differences.⁹

In our study, we did not find the earlier described relationship between previous shunt placement or a right aortic arch^{1,5} and persistent aortic dilatation. However, the few patients who had undergone palliation had had their shunt for only a few months, which probably explains why only at surgery were the root diameters significantly larger than those in the no-shunt group. No permanent effect was noted. Patients with a right aortic arch, although small in number, had a significantly larger aorta than those with a left arch at all follow-up points; however, even in these patients, a consistent decrease in the root diameters was observed. Longer follow-up is needed to determine whether this finding is sustainable or only temporary.

Study Limitations

Only 24 of the 31 patients had undergone an aortic biopsy; however, because the refusal of the parents to sign the informed consent form was completely at random, we still believe the biopsied patient sample was representative of the study population. The number of patients with a right aortic arch or previous shunt in the study cohort was small, and a larger patient group might have resulted in more pronounced differences in the aortic diameter. However, the size differences in the patients with a right aortic arch, were consistent throughout the follow-up period and, therefore, can be considered relevant. Another limitation concerned the biopsy site in the proximal ascending aorta: the histologic features at this site might differ from the histologic features at the aortic root, where the serial measurements were taken. However, because root biopsies would have been impossible without compromising the aortic cusp geometry, we opted for the biopsy site to be as close to the root as possible.

CONCLUSIONS

Infants with ToF, repaired before 6 months of age, already showed limited histologic features of aortic wall degradation at repair, mainly consisting of increased fibrosis, accumulation of ground substance in the extracellular matrix, and, occasionally, elastin fragmentation. Through prospective echocardiographic follow-up,

progressive and significant regression of the indexed aortic root diameters was demonstrated at 2 years postoperatively. Our results support the presumption that mitigation of the transaortic flow by early surgical repair of ToF will trigger a remodeling process that interrupts the progression of the limited histologic alterations of the aortic root related to increased aortic stiffness, thus preventing late aortic dilatation. Longer follow-up is needed to confirm whether this size reduction will be consistent in all patients and will ultimately lead to normalization of the aortic root diameters.

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