Cardiac Growth After Pediatric Heart Transplantation

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Background. To assess whether normal cardiac growth occurs after heart transplantation in the pediatric age group, we performed a study of 13 infants and children who underwent orthotopic heart transplantation at Stanford.

Methods and Results. The echocardiographic data from a population of 93 normal children were analyzed to determine estimates of the fifth, 25th, 50th, 75th, and 95th percentiles of the normal pediatric population. Growth curves for each of the cardiac dimensions were stratified into six classes representing each of the percentile bands, and dimensions for the 13 patients were tracked between early postoperative (early) and point of maximal follow-up (late). Results were compared by Student’s paired t test to determine whether normal growth was occurring. The mean age at transplant was 5.0±1.3 years (mean±SEM) (range, 0.4-12.8 years), duration of follow-up was 3.1±0.4 years (1.3-5.8 years), and change in body surface area was 0.24±0.03 m² (0.12-0.50 m²). Both right ventricular (RV) and left ventricular (LV) chamber dimensions were within the normal range at both early and late time points and grew normally as assessed by a lack of class changes. Early wall thickness measurements were above the 95th percentile in seven of 13 patients (LV), 12 of 13 patients (septum), and four of 13 patients (RV). Wall thickness measurements remained above normal, and there were no significant class changes at late follow-up. Histological examination in five patients showed markedly increased septal myocyte width, indicating myocyte hypertrophy. Atrial and great vessel anastomotic sites showed no evidence of obstruction by Doppler and catheterization studies.

Conclusions. These data demonstrate that normal cardiac chamber dimensional growth occurs at >3 years’ follow-up after pediatric heart transplantation. Significant LV and septal (and to a lesser extent RV) hypertrophy persists and may have implications for long-term allograft growth and function. (Circulation 1992;85:1433-1439)

KEY WORDS • transplantation, cardiac • pediatrics

Cardiac transplantation has gained increasing acceptance as a treatment modality for end-stage cardiomyopathy or noncorrectable congenital heart disease in infants and children. Encouraged by the improved survival in adult recipients after the introduction of cyclosporine in 1979, an increasing number of transplant centers have expanded their recipient selection to include younger patients. In the last half of the decade, approximately 900 pediatric heart transplants were performed at 73 centers worldwide. Improved immunosuppressive regimens have markedly increased survival in pediatric heart transplant recipients; it is now comparable to the best results in adults. These immunosuppressive drugs, however, especially prednisone, may have detrimental effects on somatic growth, a potentially serious long-term consequence for the viability of pediatric heart transplantation. It is not known whether abnormalities of cardiac allograft growth also occur in pediatric heart transplant recipients. Knowledge of the presence or severity of this potential side effect will aid in the design of immunosuppressive regimens appropriate for young transplant recipients. Thus, the purpose of the current study was to determine whether normal cardiac growth occurs after heart transplantation in the pediatric age group.

Methods

Patient Population

We studied 13 infants and children who underwent orthotopic heart transplantation at Stanford University Medical Center between September 1984 and April 1989. The mean age at transplant was 5.0±1.3 years (mean±SEM), ranging from 0.4 to 12.8 years. Initial echocardiographic evaluations (“early” follow-up) occurred at 5.7±2.8 months after transplantation and final evaluations (“late” follow-up) at 37.9±4.8 months after transplantation. The mean duration of follow-up was 3.1±0.4 years, ranging from 1.3 to 5.8 years, providing a total of 40.5 patient years. The mean change in body surface area was 0.24±0.03 m², ranging from 0.12 to 0.50...
TABLE 1. Total Daily Doses of Immunosuppressant Medications at Early and Late Follow-up

<table>
<thead>
<tr>
<th>Medication</th>
<th>Early follow-up</th>
<th>Late follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine (mg/kg/day)</td>
<td>12.5±2.1 (3.4–26.9)</td>
<td>10.5±1.2 (4.0–16.3)</td>
</tr>
<tr>
<td>Prednisone (mg/kg/day)</td>
<td>0.86±0.36 (0.18–5.05)</td>
<td>0.10±0.03 (0.00–0.25)</td>
</tr>
<tr>
<td>Azathioprine (mg/kg/day)</td>
<td>1.8±0.2 (1.0–3.2)</td>
<td>1.1±0.2 (0.0–2.2)</td>
</tr>
</tbody>
</table>

Values are mean±SEM (range).

Immunosuppression

Immunosuppression was achieved with a triple drug regimen, including cyclosporine, azathioprine, and a rapidly tapering course of prednisone over the first 3–6 months. Prednisone was then decreased further to alternate-day dosing in six patients and was eliminated entirely in three patients. The average per-kilogram doses of immunosuppressant drugs at both early and late follow-up are given in Table 1.

Normal Data

Control patient data were obtained from an echocardiographic study of a population of 93 children without heart disease performed by Rogé and colleagues13 in 1978. The raw data from that study were generously provided to us by Dr. Norman Silverman at the University of California, San Francisco. Rogé and colleagues described this population in terms of weighted regression polynomials and tolerance intervals to include 90% of the normal population with a confidence coefficient of 0.9 according to the method of Miller.14 The lower and upper tolerance limits served as conservative estimates of the fifth and 95th percentiles, respectively, and the regression line served as an estimate of the 50th percentile.

For the present study, the normal echocardiographic data were reanalyzed to determine estimates of the fifth, 25th, 50th, 75th, and 95th percentiles of the normal pediatric population with respect to body surface area. We extended Miller's method to calculate tolerance intervals to include 50% of the population so that we could also obtain estimates of the 25th and 75th percentiles. Growth curves for each of the cardiac dimensions were then stratified into six classes, representing each of the percentile bands (Figure 1, upper left panel). Class 1 represents patients falling below the fifth percentile, class 2 those patients between the fifth...
and 25th percentiles, class 3 between the 25th and 50th percentiles, class 4 between the 50th and 75th percentiles, class 5 between the 75th and 95th percentiles, and class 6 above the 95th percentile. Dimensions for each of the 13 transplant patients were then assigned to one of the six classes with respect to body surface area and tracked between early postoperative and late follow-up studies. By expressing cardiac growth as a function of body surface area, we were able to compensate for those transplant patients who have shown delayed linear growth. Although cardiac mass is linearly related to body surface area, cardiac dimensions increase as an exponential function of body surface area. In the present study, we avoided the problems inherent in a linear index by expressing all cardiac dimensions as a nonlinear function of body surface area.

**Definition of Normal Growth**

Results for each dimension were compared between early postoperative and late follow-up by Student's paired t test. Normal growth was defined as a lack of class change between early and late follow-up. Failure of growth for any dimension would be detected as a statistically significant decline in class. To verify that our method of stratifying data from the normal patient population did not itself introduce errors, we reanalyzed the raw data for one measurement, the left ventricular end-diastolic dimension, using a second statistical method to determine class divisions (the loess fit) and found no effect on our analysis of class changes. Data were also compared between early and late follow-up by comparing Z scores for each measurement with a paired Student's t test.

**Myocyte Histology**

To determine the histological characteristics of the transplanted heart, we analyzed endomyocardial biopsy specimens obtained from the right ventricle during the performance of routine surveillance biopsies. Specimens for morphometric analysis were available in five of our 13 patients. Myocyte width, an index of hypertrophy, was measured in trichrome-stained paraffin sections of right ventricular biopsies. A Microcomp morphometric system (Southern Micro Instruments, Atlanta, Ga.) was used to make a single transverse measurement passing through the middle of the nucleus in each longitudinally sectioned myocyte in which a nucleus was visible. Microscopic fields were sampled systematically, and the number of fields sampled was chosen to ensure a coefficient of error (standard error/mean) of 5% or less. Mean myocyte width was plotted versus age. Control data on right ventricular myocyte width from normal pediatric hearts were obtained from Nishikawa et al.

Right ventricular biopsies for electron microscopic morphometry were processed by the method of Rowan and Bilingham. Sections were cut from all blocks available from each patient (range, 2–5), and a total of 30 micrographs were taken, distributed evenly over all sections. Volume fractions of myofibrils and mitochondria in myocytes and the volume fraction of myocytes in myocardium were estimated by point counts using a 50-point grid overlay on each micrograph. For myofibrils and mitochondria, volume fractions were expressed per unit volume of myocyte cytoplasm minus nuclear volume (\( \frac{V_{\text{myofib}}}{V_{\text{myocyte}}} = \frac{V_{\text{nuclei}}}{V_{\text{myocytes}}} \)). Control data were obtained from biopsies of 13 donor hearts taken before implantation (age, 24±7 years). Volume fractions were compared between transplant patients and controls by an unpaired Student's t test.

Right ventricular biopsies were performed at least every 3–4 months on all patients and evaluated for the presence of cardiac rejection. If rejection was suspected by clinical or noninvasive means, it was always confirmed by endomyocardial biopsy. The number of rejection episodes was correlated with left ventricular, right ventricular, and septal wall thicknesses by regression analysis.

**Anastomatic Sites**

The atrial and great vessel anastomotic sites were interrogated by pulsed, continuous-wave, and color flow Doppler for evidence of obstruction. Routine annual posttransplant cardiac catheterization was also performed in all patients to determine the presence of obstruction at the great vessel anastomotic sites.

**Results**

**Linear Growth**

Linear growth was normal in 10 of our 13 patients. One patient was three standard deviations below normal at early follow-up but was close to the normal range at late follow-up. Two patients, both infants with relatively short follow-up periods, have shown delayed linear growth while still receiving steroids. In these two patients with poor linear growth, cardiac chamber growth occurred and was appropriate for body surface area.

**Donor–Recipient Sizes**

Because a mismatch in size between the donor and recipient could result in early alterations in the perceived rate of cardiac growth, we compared donor and recipient size by a paired Student's t test. Donors tended to be slightly older than recipients (donors, 95±29 months versus recipients, 62±16 months; \( p=0.06 \)). There were significant differences in weight between donors and recipients (donors, 25±5 kg versus recipients, 19±4 kg; \( p<0.002 \)), height (131±16 versus 106±10 cm, \( p<0.02 \)), and body surface area (0.86±0.14 versus 0.71±0.12 m²).

**Cardiac Dimensions**

Figure 1 shows cardiac chamber and aortic root dimensions plotted against body surface area for all 13 patients. Left ventricular end-systolic and end-diastolic dimensions for all 13 transplant patients fell between the fifth and 95th percentiles, and all patients have demonstrated normal chamber growth. Two patients showed increases in left ventricular end-systolic dimension of greater than one class (from the fifth to the 25th percentile to the 50–75th percentile); at late follow-up, however, both of these patients had left ventricular dimensions in the normal range. Both right ventricular end-diastolic and aortic root dimensions tended to be above the 50th percentile in transplant patients; nearly all values fell below the 95th percentile, however, and normal dimensional growth appears to be occurring, as
evidenced by the lack of a change of greater than one class in all but a single patient.

Figure 2 shows left and right ventricular and septal wall thicknesses at end diastole. At early follow-up, wall thickness measurements were above the 95th percentile in six of 13 transplant patients for the left ventricle, 12 patients for the septum, and four patients for the right ventricle. At late follow-up, wall thickness measurements remained above normal in seven patients for the left ventricle, nine patients for the septum, and six patients for the right ventricle.

Class Changes

A summary of the dimensional data is shown in Figure 3, with early postoperative and late follow-up data stratified by class on the left axis and by percentile on the right axis. Left ventricular dimensions have mean class ranks close to the 50th percentile, the right ventricular dimension averages at the 75th percentile, and that of the aortic root averages close to the 95th percentile. Comparison of early and late follow-up class ranks showed that there were no significant class shifts, indicating normal growth. The magnitude of the cardiac hypertrophy can be appreciated from the high mean class ranks for the wall thickness measurements. There were no significant differences in class rank for any of these dimensions, indicating that the degree of increased wall thickness kept pace with somatic growth. Evaluation of dimensional changes by comparison of Z scores also demonstrated no significant changes for any variable between early and late follow-up periods (Table 2).

Histology

To determine whether the increase in wall thicknesses we have described echocardiographically represents true myocyte hypertrophy, we analyzed serial right ventricular endomyocardial biopsies in five transplant patients. Mean myocyte width was increased in all patients compared with normal controls (Figure 4).

Figure 3. Bar graph shows summary of class changes between early postoperative and late follow-up data. Class ranks are shown on the left axis and their representative percentiles on the right axis. None of the changes were statistically significant by Student's paired t test. LVESD, left ventricular end-systolic dimension; LVEDD, left ventricular end-diastolic dimension; RVDD, right ventricular diastolic dimension; AOD, aortic diastolic dimension; LVPWD, left ventricular wall thickness; SEPTD, septal wall thickness; RVAWD, right ventricular wall thickness at end diastole.
TABLE 2. Comparison of Z Scores Between Early and Late Follow-up for Each Cardiac Dimension

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Early follow-up</th>
<th>Late follow-up</th>
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<tbody>
<tr>
<td>LVEDD</td>
<td>-0.26±0.22</td>
<td>-0.22±0.20</td>
</tr>
<tr>
<td>LVESD</td>
<td>-0.31±0.30</td>
<td>0.01±0.18</td>
</tr>
<tr>
<td>RVEDD</td>
<td>0.60±0.39</td>
<td>0.37±0.24</td>
</tr>
<tr>
<td>AOD</td>
<td>1.35±0.27</td>
<td>0.90±0.19</td>
</tr>
<tr>
<td>LVPWD</td>
<td>2.53±0.37</td>
<td>1.89±0.25</td>
</tr>
<tr>
<td>SEPTD</td>
<td>3.17±0.31</td>
<td>2.68±0.33</td>
</tr>
<tr>
<td>RVAWD</td>
<td>1.45±0.38</td>
<td>1.56±0.57</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; RVEDD, right ventricular end-diastolic dimension; AOD, aortic diastolic dimension; LVPWD, SEPTD, and RVAWD, left ventricular, septal, and right ventricular wall thicknesses at end diastole, respectively.
p =NS by paired Student's t test.

Myocyte width was greater than normal whether indexed to donor or recipient age and remained increased over time periods ranging from 1.1 to 4.8 years (mean, 2.48±0.6 years) after transplant. Electron microscopy demonstrated that myofibril and mitochondrial volume fractions were not different from the controls (Table 3).

Anastomotic Sites

Doppler studies showed no evidence of significant obstruction at atrial suture sites, and cardiac catheterization studies showed no evidence of obstruction at great vessel anastomotic sites in any of our patients (Table 4).

Hypertension and Rejection History

To determine the potential role of immunosuppressive drug-related hypertension in producing myocardial hypertrophy, we evaluated blood pressures in all 13 transplant patients. Systolic and diastolic blood pressures were 119±5 mm Hg (95–160 mm Hg) and 68±5 mm Hg (40–92 mm Hg), respectively, at early follow-up and 111±5 mm Hg (90–130 mm Hg) and 73±3 mm Hg (58–90 mm Hg) at late follow-up. Most pediatric transplant recipients developed moderate hypertension, which was well controlled with antihypertensive medications, including captopril (two patients), diuretics (seven patients), hydralazine or prazosin (six patients), diltiazem (three patients), and a β-adrenergic blocker (one patient). At the time of the late follow-up evaluation, only one patient had a systolic blood pressure that was above normal for age.

There were a total of 24 episodes of rejection in our 13 patients over the course of the study. There were 1.8±0.4 (range, 0–5) rejection episodes per patient, with two patients never experiencing an episode of rejection, and eight patients having more than one episode. Only three patients had rejection before the early echocardiographic dimensional measurements. Regression analysis revealed no relation between number of episodes of rejection and left ventricular wall thickness (r²=0.04), septal thickness (r²=0.0003), or right ventricular wall thickness (r²=0.31).

Discussion

Improved immunosuppressive regimens, starting with the introduction of cyclosporine in 1979, have allowed the proliferation of heart transplantation in the pediatric age group.2-4,7-9 The most successful of these regimens have used triple drug therapy, combining cyclosporine with prednisone and azathioprine.1,23,24 These immunosuppressive agents, prednisone in particular, have detrimental effects on linear growth.10-12 Although several studies have described echocardiographic and histological changes in the mature heart after transplantation,25,26 it has not been previously determined whether normal growth occurs in the immature cardiac allograft.

In this study of 13 pediatric heart transplant recipients followed for an average of 3 years, we have demonstrated that even in the presence of delayed linear growth, normal cardiac chamber growth occurs. Because ventricular systolic and end-diastolic dimensions may be more dependent on volume loading conditions than on increases in cell size, our short-term results are not surprising. Whether this pattern of growth can be maintained over longer periods, for example, as infant allograft recipients reach adulthood, remains to be determined. We have demonstrated normal chamber growth in one of our younger patients.

FIGURE 4. Graph of mean myocyte width obtained from right ventricular endomyocardial biopsies in five pediatric patients plotted vs. age. Large circles with error bars represent mean±1 SD for normal right ventricles from pediatric patients from the study by Nishikawa et al.20 Myocyte width was increased above normal levels in all patients and remained increased for follow-up periods of 1.1–4.8 years after transplant.

TABLE 3. Histological Analysis of Endomyocardial Biopsy Specimens From Pediatric Heart Transplant Patients Compared With Controls

<table>
<thead>
<tr>
<th></th>
<th>Transplant patients (n=5)</th>
<th>Controls (n=13)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myofibril volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fraction</td>
<td>45.2±16.0</td>
<td>47.7±0.8</td>
</tr>
<tr>
<td>Mitochondrial volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fraction</td>
<td>24.2±2.0</td>
<td>25.2±0.9</td>
</tr>
<tr>
<td>Myocyte width</td>
<td>12.9±0.5</td>
<td>7.6±0.3†</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*Control data obtained from pretransplant biopsies taken from the right side of the septum in 13 donors (age, 24±7 years).

†p≤0.0001 by Student's t test.
(transplanted at age 2.8 years) for as long as 5.8 years after transplant. Furthermore, early data from Loma Linda suggest the potential for normal cardiac chamber growth in infant heart transplant recipients. In our study, it is possible that the size discrepancy between donors and recipients could have resulted in an exaggeration of initial dimensions, and this appeared to be the case in several patients in whom both aortic root and right ventricular chamber dimensions decreased markedly at late follow-up (Figure 1). It is unlikely that early rejection could have given the false impression of cardiac growth (by causing a decrease in compliance and ventricular volume at early follow-up), because only three of our patients experienced rejection before this time. Serial studies have also shown no abnormal contraction of ventricular volume at any of the early time points. Although our results show that atrial and great vessel anastomotic sites have not developed obstruction, recoarctation has been reported in infants after transplantation for the hypoplastic left heart syndrome. None of the patients in the present series received their allografts for hypoplastic left heart syndrome.

Despite normal growth of cardiac chamber size, significant left ventricular and septal and, to a lesser extent, right ventricular hypertrophy occurs in our transplant recipients. This increase in wall thickness was not present on day 1 or 2 after transplant but developed as early as the first week after transplant. Similar increases in wall thickness have been described previously in both adult and pediatric heart transplant recipients. In our patients, increased wall thickness, particularly of the left ventricle and septum, persisted to a maximum of 5.8 years after transplant. Unlike previous echocardiographic studies, we also present histological evidence demonstrating myocardial cell hypertrophy, which also persists at long-term follow-up (Figure 4). These histological findings are consistent with studies in adult cardiac allograft recipients; unlike adults, however, we did not find a reduction in myofibril volume fraction. Reduced myofibril volume fraction is a characteristic of severe, pathological hypertrophy, so the maintenance of normal myocyte volume fraction in our pediatric patients is suggestive of either normal cardiac growth or physiological, rather than pathological hypertrophy. This finding may have important implications for the preservation of long-term contractile function; this issue was not addressed in the present study. Although the donor hearts used for the control group were older than those of our transplant patients, previous studies have shown that myofibril volume fraction increases with postnatal age and that adult concentrations of myofibrils are reached shortly after birth.

Previous investigators have invoked several mechanisms to explain this persistent hypertrophy, including exposure to elevated circulating catecholamines after cardiac denervation, chronic volume overload, and graft ischemia. Our results, however, do not show evidence of chronic volume overload in pediatric patients. Similarly, mechanical factors related to size discrepancies between the recipient and donor are unlikely cause of hypertrophy, because the increase in wall thickness is not present in the first few days after transplant and because allograft hypertrophy is also common in adults, where size discrepancies between donor and recipient are not as significant. It is also unlikely that immunosuppression-related systemic hypertension is the mechanism, because hypertrophy has been found to be equally common in both hypertensive and normotensive adult transplant recipients. Although all of our patients were taking medication for systemic hypertension, systolic and diastolic blood pressures were well controlled. Similarly, there was no correlation between the number of rejection episodes and cardiac wall thickness in our patients. In adults, Rowan and Billingham have shown a slight increase in collagen content related to frequency of rejection episodes, but no relation to myocyte hypertrophy.

In summary, the present study demonstrates that normal cardiac chamber growth occurs at >3 years average follow-up after pediatric heart transplantation. Atrial and great vessel anastomotic sites have not as yet developed significant obstruction. However, the significant left ventricular and septal and, to a lesser extent, right ventricular hypertrophy noted shortly after transplant persists.

Acknowledgments
The authors would like to thank Dr. Norman Silverman for providing the echocardiographic data on normal children and Dr. Byron Brown for his assistance with the statistical methods.

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