Clinical Determinants of Ventricular Sympathetic Reinnervation After Orthotopic Heart Transplantation

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Background—It has been demonstrated that ventricular sympathetic reinnervation after cardiac transplantation improves exercise performance. The extent of reinnervation increases with time but is variable. Little is known about other influencing factors.

Methods and Results—Seventy-seven nonrejecting transplant recipients were cross-sectionally studied by PET with the catecholamine analogue C-11 hydroxyephedrine at 4.8±3.5 years after transplantation. Results were compared with history-derived parameters related to recipient’s clinical course before, during, and after surgery; donor characteristics; and immunogenetics. Partial reinnervation was observed in 52 patients (extent, 21±16% of left ventricle). Complete denervation was found in 25 patients at various times after transplantation. Reinnervation extent correlated with time after surgery ($r=0.387$, $P<0.001$) but also inversely with donor age ($r=-0.309$, $P=0.006$) and recipient age ($r=-0.243$, $P=0.032$). Maximal hydroxyephedrine retention correlated inversely with frequency of rejection episodes ($r=-0.267$, $P=0.019$), was reduced when aortic complications occurred perioperatively (9 patients), and correlated inversely with aortic cross-clamp time ($r=-0.331$, $P=0.006$). Other parameters were not associated with reinnervation. Patients were surveyed for clinical complications over >12 months after PET (until 7.3±4.2 years after transplantation), but significant effects of reinnervation on outcome were not observed.

Conclusions—The present data suggest that sympathetic reinnervation after cardiac transplantation is not simply a function of time. Reinnervation is more likely with young age, fast and uncomplicated surgery, and low rejection frequency. Despite few effects on prognosis in otherwise healthy recipients, improved understanding of clinical determinants may contribute to enhance allograft reinnervation and thereby augment exercise capacity in the future. (Circulation. 2002; 106:831-835.)

Key Words: nervous system, sympathetic ▪ transplantation ▪ tomography

Sympathetic nerve fibers are surgically disrupted at cardiac transplantation, causing complete denervation of the transplanted heart. The occurrence of subsequent partial reinnervation of the left ventricle has been demonstrated in various studies,1–6 and the physiological importance of this phenomenon has been supported by description of beneficial effects on regulation of myocardial blood flow,7 metabolism,8,9 and exercise performance.10,11 Because of the increasing evidence that restoration of sympathetic innervation is advantageous for transplant recipients, approaches to support reinnervation are of interest, and a more detailed understanding of clinical determinants of reinnervation is desirable.

It is well known that reinnervation increases with time after surgery,1,2,4–6,12 but observations of interindividual heterogeneity6,12 and the regionally incomplete pattern6,13 suggest that additional determinants are involved. A few studies suggested other influencing factors for the development of reinnervation, such as the type of disease before transplantation,4 age of recipient,12 or age of donor,10 but identification of these parameters was not the major purpose of these studies, and the numbers of patients were too small to draw definite conclusions. Thus, little is known about potential determinants of allograft reinnervation other than time after transplantation.

We therefore combined all transplant recipients previously investigated by PET at our institute into the largest group of patients assessed for ventricular reinnervation to date. A wide variety of clinical parameters were collected for multivariate analysis to identify their influence on extent and intensity of sympathetic nerve terminal reappearance. Finally, in addition to identification of clinical determinants, the impact of reinnervation on outcome was evaluated for the first time.

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**Methods**

**Patients**
Between May 1994 and September 2000, a total of 77 cardiac transplant recipients (11 women, 66 men) underwent PET for assessment of sympathetic reinnervation at the Nuklearmedizinische Klinik der Technischen Universität München. Patients were prospectively selected from a large group of >500 patients who had undergone transplantation since 1981 at the Herzchirurgische Klinik der Ludwig-Maximilians-Universität München to participate in projects aiming at characterization of allograft reinnervation and its physiological relevance. \(^{6,10,12}\) Selection was based on availability, willingness to participate, and absence of acute rejection, clinically significant transplant vasculopathy (defined by absence of significant coronary stenoses at angiography and/or normal dobutamine stress echocardiography), allograft dysfunction, diabetes mellitus, other symptomatic noncardiac diseases, and medication known to interfere with presynaptic catecholamine uptake. Twenty-three of the patients had undergone transplantation because of ischemic cardiomyopathy and the other 54 because of idiopathic cardiomyopathy. The mean interval between transplantation and PET was 4.8 ± 3.5 years (range 0.5 to 15 years). Clinical parameters characterizing recipient, donor, and the surgical procedure (atrial anastomosis according to Lower/Shumway in all cases) are summarized in Table 1. Donor/recipient human leukocyte antigen (HLA) compatibility ranged from 0 (1 patient) to 6 (8 patients) HLA mismatches, with the majority having 4 (22 patients) or 5 (24 patients) mismatches. After transplantation, immunosuppression was based on cyclosporine in 53 patients and tacrolimus in 24. The number of rejection episodes before PET ranged from 0 to 6 (8 patients). All individuals signed written informed consent forms approved by the ethics committee of the Technischen Universität München before performance of PET imaging.

**Positron Emission Tomography**
PET was performed as described previously\(^ {6,9,10,12}\) with an ECAT 951, EXACT, or EXACT HR + scanner (CTI/Siemens). Briefly, after acquisition of a scan and quantitative assessment of myocardial perfusion with \(^{13}N\)-ammonia or \(^{15}C\)acetate, 600 MBq of the catecholamine analogue \(^{13}C\)hydroxyephedrine (HED) was injected intravenously, and a dynamic imaging sequence was acquired. Volumetric sampling of myocardial radioactivity was used to calculate polar maps of myocardial HED retention (defined as cardiac activity at 40 minutes divided by the integral of the arterial input function). On the basis of previously defined mean and SD values in denervated patients early after surgery,\(^ {2,12}\) segments with a retention of <7% per minute were defined as denervated according to previous studies.\(^ {6,10,12}\)

**Clinical Follow-Up**
All patients underwent routine follow-up that consisted of clinical evaluation, echocardiography, coronary angiography, and endomyocardial biopsy. Patients were surveyed for >12 months after PET, until 7.3 ± 4.2 (range 1.5 to 19) years after transplantation. Events were recorded and summarized as cardiac hard events (cardiac death or retransplantation) or soft events (intervention for transplant vasculopathy or episodes of allograft failure).

**Statistical Analysis**
Data were analyzed with StatView version 5.0 software (SAS Institute). Values are expressed as mean ± SD. Differences between groups were assessed by unpaired t test or (for multiple comparisons) by 1-way ANOVA with post hoc Fisher’s protected least significant differences test. Simple linear regression was used to describe relations between pairs of continuous variables. Multivariate stepwise linear regression was applied to identify independent determinants of ventricular reinnervation. The effect on patient outcome was investigated by Kaplan-Meier survival analysis and log-rank test. A 2-sided probability value of <0.05 was defined as significant.

**Results**

**Sympathetic Reinnervation**
Myocardial perfusion at rest was homogeneous in all patients. Perfusion defects were not observed, confirming myocardial integrity at the time of PET imaging.

Specific HED retention >7% per minute, indicating sympathetic reinnervation, was found in 52 patients. In those, the reinnervated area comprised 21 ± 16% of the left ventricle, mainly located in the basal anterior and septal wall. Complete denervation was found in 25 patients until 15 years after transplantation but most frequently within the first 18 months (Figure 1). Maximal left ventricular myocardial HED retention as a measure of intensity of innervation ranged from 3.4% to 20.7% per minute (mean 8.7 ± 3.6% per minute). Significant correlation with time after transplantation was present for maximal retention (r = 0.393, P < 0.001) and extent of reinnervation (r = 0.387, P < 0.001).

**Reinnervation and Recipient Characteristics**
A mild but significant inverse correlation was observed between age of the recipient at transplantation and extent of reinnervation (r = −0.243, P = 0.032). Additionally, maximal

**Table 1. Characteristics of Transplant Recipients, Donors, and Surgical Procedure**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Patients Available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at transplantation, y</td>
<td>49.6 ± 9.4</td>
<td>23–60</td>
<td>100%</td>
</tr>
<tr>
<td>Body mass index at transplantation, kg/m²</td>
<td>23.7 ± 2.2</td>
<td>18.3–28.7</td>
<td>97% (75/77)</td>
</tr>
<tr>
<td>Ejection fraction before transplantation, %</td>
<td>22 ± 9</td>
<td>10–45</td>
<td>66% (51/77)</td>
</tr>
<tr>
<td>Duration of heart failure, y</td>
<td>5.9 ± 6.1</td>
<td>0.1–28</td>
<td>79% (61/77)</td>
</tr>
<tr>
<td>Donor characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>30.4 ± 12.0</td>
<td>13–59</td>
<td>100%</td>
</tr>
<tr>
<td>Difference between recipient and donor, y</td>
<td>19 ± 13</td>
<td>(−17)–44</td>
<td>100%</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.8 ± 2.4</td>
<td>15.6–27.7</td>
<td>86% (66/77)</td>
</tr>
<tr>
<td>Difference between recipient and donor, kg/m²</td>
<td>0.2 ± 3.2</td>
<td>(−7.4)–7.6</td>
<td>84% (65/77)</td>
</tr>
<tr>
<td>Transplant surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic cross-clamp time, min</td>
<td>58 ± 20</td>
<td>32–166</td>
<td>88% (68/77)</td>
</tr>
<tr>
<td>Duration of allograft cold ischemia, min</td>
<td>160 ± 51</td>
<td>73–305</td>
<td>79% (61/77)</td>
</tr>
</tbody>
</table>
HED retention was higher in patients undergoing transplantation because of idiopathic cardiomyopathy than in those with prior ischemic cardiomyopathy (9.4±3.3% per minute versus 7.2±4.0% per minute, \(P=0.016\)). Duration of disease and left ventricular ejection fraction before transplantation did not correlate with parameters of reinnervation, and neither did weight, height, or body mass of recipients.

Reinnervation and Donor Characteristics
Donor age was significantly inversely correlated with extent of reinnervation (\(r=−0.309, P=0.006\)) and maximal HED retention (\(r=−0.315, P=0.005\); Figure 2). Other available donor characteristics (Table 2) were not associated with reinnervation.

Reinnervation and Transplant Surgery
Perioperative complications with regard to aortic anastomosis (severe size mismatch, \(n=3\); aortic bleeding requiring rethoracotomy, \(n=4\)) or ventricular function (asystole, \(n=1\); right heart failure, \(n=1\)) occurred in 9 patients. In this subgroup, both maximal HED retention (6.3±2.4% per minute versus 9.1±3.7% per minute, \(P=0.034\)) and extent of reinnervation (3.1±4.9% versus 15.6±16.9% of left ventricle, \(P=0.032\)) were significantly reduced. Aortic cross-clamp time was inversely correlated with maximal retention (\(r=−0.331, P=0.006\)) and extent of reinnervation (\(r=−0.284, P=0.019\)). Time of cold allotraft ischemia was not correlated.

Reinnervation and Immunogenetic Issues
Neither the sex of recipient or donor nor the presence of a sex mismatch between recipient and donor (\(n=19\)) had an effect on sympathetic reinnervation. Donors and recipients were all matched for ABO characteristics. An Rh mismatch (recipient negative, donor positive) was identified in 11 patients, but reinnervation did not differ from matched patients. No significant differences of reinnervation were observed between subgroups with different degrees of overall HLA mismatch (Figure 3) or mismatch in HLA-A, -B, and -DR loci. Additionally, no differences between cyclosporine- or tacrolimus-based immunosuppression were observed. Cytomegalovirus infection of the recipient (\(n=18\)) or donor (\(n=29\)) was also not associated with impaired reinnervation. The annual frequency of rejection episodes before PET, however, showed a significant inverse correlation with maximal HED retention (\(r=−0.267; P=0.019\)).

Multivariate Analysis
All clinical characteristics that were tested for association with reinnervation are summarized in Table 2. Those that correlated significantly at univariate analysis (time after transplantation, age of recipient, age of donor, disease before transplantation, presence of surgical complications, aortic cross-clamp time, and frequency of rejection) were entered in a multivariate stepwise linear regression analysis to identify independent determinants of maximal myocardial HED retention. In the final model, only age of donor, aortic cross-clamp time, and frequency of rejection were independently associated.

Reinnervation and Outcome
One patient was lost to follow-up after performance of PET, and 3 died of noncardiac disease (malignancies). In the remaining 73 transplant recipients, hard events were observed in 6 (4 cardiac deaths, 2 retransplantations), soft events in 10 (7 revascularizations, 3 episodes of allograft failure), and overall cardiac events in 12. Survival analysis did not reveal a significant association between presence/absence of reinnervation and occurrence of hard events or overall events (Figure 4).

Discussion
Postganglionic sympathetic nerve fibers, which travel from the stellate ganglia along the arterial structures to the myocardium, are transected at cardiac transplantation, resulting in axonal degeneration and rapid depletion of the neurotransmitter norepinephrine from nerve terminals, and thus complete denervation. Because regrowth of nerve fibers takes time, it is not surprising that sympathetic reinnervation is observed more frequently later after transplantation. Interestingly, at multivariate analysis, time after transplantation was not an independent determinant of reinnervation in the present study. Reinnervation generally remains incomplete, and some patients remained completely denervated even late after transplantation, which suggests the influence of other parameters. Molecular mechanisms underlying allograft reinnervation are poorly understood. Although they cannot be clarified in detail from the present study, identification of some additional clinical determinants gives further insights into the process of reinnervation.

From studies in peripheral nerves, it is known that neuronal regeneration is dependent on neurotrophins, such as neuronal growth factor, which are produced and released by target tissue. Aging has been suggested to be associated with
reduced availability of target-derived neurotrophic factors.16 This observation may serve as an explanation for the relationship of sympathetic reinnervation with age of donor and (to a lesser degree) recipient. Reduced availability and synthesizing capacity of neurotrophins may also explain the lower degree of reinnervation in cases with more frequent rejection episodes.

A variety of immunogenetic parameters, on the other hand, were not directly associated with sympathetic reinnervation. Although a trend toward higher myocardial catecholamine retention was observed for higher degrees of HLA matching, differences were not statistically significant. No effects of sex, cytomegalovirus infection, or the immunosuppressive regimen were identified. The direct influence of immunogenetic factors on reinnervation thus appears to be limited, although they may indirectly affect innervation when associated with higher rejection frequency.17

Because surgical dissection results in axonal degeneration, sympathetic nerve fibers need to regrow along arterial structures to reach the allograft as their target organ. Extensive areas of scar tissue or other morphological alterations along the path of regrowth may thus impair reappearance of nerve terminals in the myocardium. This is confirmed by less extensive reinnervation in patients with aortic complications at transplant surgery and the significant inverse correlation with aortic cross-clamp time in the present study. Hence, the surgical procedure appears to be another factor that may influence reinnervation. The observation of more intense reinnervation in patients receiving transplants for dilated compared with ischemic cardiomyopathy, although not an independent determinant at multivariate analysis, may also be explained in this context, because regrowth along sclerotic aorta and other vessels may be more difficult. The present finding is in contrast to a previous study that reported lower uptake of radiolabeled metiodobenzylguanidine for patients undergoing transplantation because of dilated cardiomyopathy, albeit in a much smaller and thus statistically less reliable group.5 Patients with ischemic heart disease are thought to have a higher incidence of transplant vasculopathy and less beneficial outcome after transplantation than those with dilated cardiomyopathy.18 Interrelations between development of transplant vasculopathy and reinnervation cannot be investigated conclusively from the present study group, because patients with known clinically relevant graft vessel disease were not included. Other studies in smaller groups, however, have suggested a negative association between vasculopathy and ventricular reinnervation.19

The physiological importance of sympathetic reinnervation for the transplanted heart has been demonstrated in several studies showing, for example, effects on the sensation of chest pain,3 regulation of myocardial blood flow,7 chrono- tropic responsiveness,20 and ventricular function and exercise performance.10 It is therefore tempting to speculate about potential beneficial effects on outcome after transplantation. This was evaluated for the first time in the present study, but results need to be interpreted with caution. Analysis was performed retrospectively, and the patient group consisted of healthy, symptom-free recipients with an uncomplicated course, which is reflected by substantially higher survival rates than for cardiac transplant recipients in general.21 Although major outcome differences could not be detected in this selected group, prospective trials with larger patient groups or higher event rates will be necessary in the future for a more detailed analysis of the contribution of reinnervation to patient outcome after transplantation.

In conclusion, although survival analysis in this selected group did not suggest a major impact of reinnervation on outcome, improved understanding of determinants of reinnervation may still be clinically advantageous because of the

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**TABLE 2. Parameters Tested for Association With Reinnervation**

<table>
<thead>
<tr>
<th>Recipient Related</th>
<th>Donor Related</th>
<th>Surgery Related</th>
<th>Immunogenetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of PET after HTX*</td>
<td>Age*†</td>
<td>Allograft cold ischemia</td>
<td>Recipient sex</td>
</tr>
<tr>
<td>Weight at HTX</td>
<td>Age difference</td>
<td>Aortic cross-clamp time*†</td>
<td>Donor sex</td>
</tr>
<tr>
<td>Height at HTX</td>
<td>Weight</td>
<td>Perioperative aortic complications*</td>
<td>Sex mismatch</td>
</tr>
<tr>
<td>Age at HTX*</td>
<td>Height</td>
<td></td>
<td>Rh mismatch</td>
</tr>
<tr>
<td>Body mass at HTX</td>
<td>Body mass</td>
<td></td>
<td>HLA-A mismatch</td>
</tr>
<tr>
<td>Ejection fraction before HTX</td>
<td>Body mass difference</td>
<td></td>
<td>HLA-B mismatch</td>
</tr>
<tr>
<td>Disease type*</td>
<td>CMV infection</td>
<td></td>
<td>HLA-DR mismatch</td>
</tr>
<tr>
<td>Duration of disease</td>
<td></td>
<td></td>
<td>Overall HLA mismatch</td>
</tr>
<tr>
<td>CMV infection</td>
<td></td>
<td></td>
<td>Type of immunosuppression</td>
</tr>
<tr>
<td>Outcome after HTX</td>
<td></td>
<td></td>
<td>Rejection frequency*†</td>
</tr>
</tbody>
</table>

HTX indicates heart transplantation; CMV, cytomegalovirus.

*Significant at univariate analysis.
†Independent at multivariate analysis.

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Figure 3. Maximal myocardial HED retention in subgroups with different degrees of HLA mismatch.
previously demonstrated beneficial functional effects.10,11 In a previous study in 29 transplant recipients (who are a subgroup of the present study group), we clearly identified improved chronotropic and inotropic responsiveness to exercise in reinnervated recipients that was associated with higher exercise capacity and a trend toward higher physical activity in daily life.10 Several clinical determinants of ventricular reinnervation other than time after transplantation were identified in the present study. Surgeons can expect more rapid and intense reinnervation of an allograft if hearts of younger donors are chosen and if the procedure of transplantation is fast and uncomplicated. Additionally, transplant recipients who are younger and who had dilated nonischemic cardiomyopathy before surgery, as well as those who had few rejection episodes, can expect to have a higher likelihood of future reinnervation and thus a higher likelihood for sustained improvement of exercise capacity.

References
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