Pulmonary Diffusion Abnormalities in Heart Transplant Recipients*  
Relationship to Cytomegalovirus Infection

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Lung function of patients with heart failure is characterized by a variety of changes proposed as being due to passive congestion, secondary pulmonary fibrosis, and/or recurrent pulmonary emboli. A diffusion impairment thought to be due to cyclosporine has also been noted in patients following heart transplantation. Similar changes of unclear origin have been observed in renal transplant recipients. The objective of this study was to determine the extent to which lung function changes are reversible by cardiac transplantation and relate changes to the status of the recipients lung in the presence of possible vascular, iatrogenic, immune, or infectious injury. We analyzed the data of 22 patients who underwent lung function testing before and after heart transplantation and correlated changes to hemodynamic change, episodes of rejection, concentration of cyclosporine, and cytomegalovirus infection. Despite excellent graft function, the carbon monoxide transfer factor deteriorated to a mean of 57 percent of predicted postoperatively. The fall in diffusion factor did not correlate with episodes of cardiac rejection, cyclosporine levels, or hemodynamic status. In those patients who had serologic evidence of cytomegalovirus infection, the reduction in transfer factor was greater compared to those without infection despite a normal chest radiograph. The effects of cardiopulmonary bypass were unlikely to have been responsible for the abnormalities as lung function was assessed at a mean of 14 months after surgery. In heart transplant recipients, a change in diffusion capacity may represent an additional marker for cytomegalovirus infection and reflect infectious/immune injury late following surgery. (Chest 1993; 104:1085-99)

Methods

Data were available from 22 cardiac transplant patients (21 male; mean age, 50 years; range, 17 to 60 years; 19 exsmokers) who had lung function studies completed before and following surgery. Before surgery, all of the patients had severe congestive cardiac failure (New York Heart Association grade 3 or 4). Lung function testing was carried out at an initial transplantation assessment an average 6.7 months (1 to 26 months) before surgery and an average 14 months (1 to 42 months) after surgery. Six patients had been treated with amiodarone prior to surgery and one patient had suffered a pulmonary embolus (proved by ventilation perfusion scan). No other patient had evidence of pulmonary emboli, it being a relative contraindication to transplantation.

Lung function was measured as follows: forced expiratory volume in 1 s (FEV1); forced vital capacity (FVC); residual volume (RV); functional residual capacity (FRC) (Gould Pulmonet 111 wet spirometer); maximal inspiratory and expiratory flow volume loop; total lung capacity (TLC) (Jaeger Body Plethysmograph). For single breath carbon monoxide diffusion (Dco) and gas transfer coefficient (Kco) (PK Morgan transfer test), at least four reproducible and technically satisfactory recordings were obtained. The largest value was selected for analysis. The transfer test was calibrated for each patient and the study was performed by an experienced qualified physiologist technician. Both the expired and inspired gas were subjected to analysis. All Dco values were corrected for hemoglobin although no patient was anemic. Predicted values were obtained from the European Community for Coal and Steel predicted equations. Each patient was capable of holding his/her breath for 10 s and a variable was deemed abnormal if less than 20 percent predicted. Quality control (lung function studies are performed by a respiratory physiology technician) is carried out on a weekly basis at this regional respiratory physiology center.

Pulmonary artery pressure (PAP), pulmonary capillary wedge pressure (PCWP), and pulmonary vascular resistance (PVR) were collected from the postoperative assessment data. Postoperatively
Table 1—Lung Function and Hemodynamic Data Before and After Transplantation

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Transplant†</th>
<th>p</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predicted</td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>FEV₁, L</td>
<td>3.43</td>
<td>2.68 (0.14)</td>
<td>2.77 (0.14)</td>
<td>NS</td>
</tr>
<tr>
<td>VC, L</td>
<td>4.32</td>
<td>3.43 (0.17)</td>
<td>3.47 (0.16)</td>
<td>NS</td>
</tr>
<tr>
<td>TLC, L</td>
<td>5.81</td>
<td>5.35 (0.22)</td>
<td>5.32 (0.18)</td>
<td>NS</td>
</tr>
<tr>
<td>Vmax50, L/s</td>
<td>5.3</td>
<td>4.01 (0.27)</td>
<td>4.20 (0.53)</td>
<td>NS</td>
</tr>
<tr>
<td>Dco</td>
<td>9.7</td>
<td>7.80 (0.40)</td>
<td>5.60 (0.30)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Kco</td>
<td>1.7</td>
<td>1.60 (0.07)</td>
<td>1.20 (0.30)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>22.3</td>
<td>22.0 (2.20)</td>
<td>9.20 (1.20)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MPAP, mm Hg</td>
<td>32.9</td>
<td>32.7 (2.70)</td>
<td>18.4 (1.30)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Dco/Kco units in mmol/min/kPa. FEV₁ = forced expiratory volume in 1 s; VC = vital capacity; TLC = total lung capacity; Vmax50 = midexpiratory flow; Dco = diffusing capacity for carbon monoxide; Kco = gas transfer coefficient; PCWP = pulmonary capillary wedge pressure; MPAP = mean pulmonary artery pressure.
†Mean (SEM).

PAP, PCWP were recorded at the time of right ventricular endomyocardial biopsy and a chest radiograph. Pulmonary function tests were completed on the same day prior to the heart biopsy being performed.

Histologic rejection was graded according to international classification. The total duration of rejection in weeks from the time of transplantation was recorded for each patient. The severity of rejection on the same day as lung function testing was also documented. Whole blood trough cyclosporine concentration was measured by a nonspecific polyclonal radioimmunoassay (Abbott). The mean cyclosporine concentration was calculated for each patient by the sum of all recorded levels divided by the number of observations.

Cytomegalovirus-specific IgG and IgM antibodies were measured by an ELISA test.* Patients were screened for CMV-specific IgG prior to transplantation; donor serum samples were also tested whenever available. Following transplantation, recipients were tested for CMV-specific IgG and IgM routinely at the same time as right ventricular biopsy. Active CMV infection was defined as the presence of a significant serologic change for CMV-specific IgM antibody (a fourfold rise in IgM) in the absence of a significant serologic change for CMV-specific IgG antibody (a fourfold rise in IgG). The presence of CMV disease was defined as an unexplained pyrexia with or without a leukopenia or an elevation of serum transaminase level. Also, no patient had CMV disease (ie, no evidence of pneumonitis or other end-organ damage). All patients received acyclovir, 200 mg three times a day as prophylaxis against herpes simplex for 3 months following transplantation. It is unlikely that this modified the natural history of CMV in our patients. In those two CMV-seronegative patients who received an organ from a seropositive donor, hyperimmunoglobulin was given as prophylaxis, as detailed elsewhere. No other prophylaxis was administered to seropositive recipients during the period of time studied.

Immunosuppression was induced using azathioprine, 4 mg/kg preoperatively, and 1 g of methylprednisolone preoperatively. Rabbit antithymocyte globulin was given on day 1 for 3 days using a dose of 1.5 mg/kg. Maintenance immunosuppression was achieved using a triple-therapy regimen of the following: cyclosporine, 4 mg/kg/d; azathioprine, 1 to 2 mg/kg/d; and prednisolone, 0.1 to 0.2 mg/kg/d.

All lung function measurements before and after transplantation were compared using paired Student's t test. Linear regression analysis and correlation coefficient were used to assess the relationship between the posttransplant Dco and Kco, and hemodynamic measurements, rejection status, and the cyclosporine levels. Comparison between CMV-seropositive patients and CMV-seronegative patients was done with the unpaired Student's t test. Significance was set at the 5 percent level.

RESULTS

The mean values of FEV₁, VC, TLC, midexpiratory flow (Vmax50), Dco, and Kco prior to and following surgery are given in Table 1. Before transplantation, there was a minor reduction in the mean vital capacity (percent predicted) and mean Dco (percent predicted) in the presence of a normal mean diffusion coefficient (percent predicted). Nine of 22 (36 percent) of the patients had an abnormal Dco prior to transplantation (Fig 1). Of the five patients with an abnormal Kco before surgery, two had been receiving amiodarone and one of these required warfarin following a pulmonary embolus. There was no improvement in diffusion capacity in those patients whose amiodarone therapy was discontinued following surgery.

The mean Dco and the mean Kco showed a significant fall following transplantation (p<0.0001) (Table 1) with 18 of 22 patients having a fall in Dco of greater than 10 percent (Fig 1 and 2). Two of these patients had a normal Dco both before and following transplantation. Six patients maintained a normal Kco following transplantation (Fig 3 and 4). As expected, the PCWP and PAP measurements demonstrated a significant improvement following transplantation (Table 1). There was no correlation between the Dco and Kco and any preoperative hemodynamic variable (PCWP,
Group B. Patients with no change in CMV serology.

PAP, and PVR). The difference between the postoperative PAP and PCWP (the transpulmonary gradient, PAP-PCWP) did not correlate with either the postoperative Kco or Dco. Neither the cyclosporine level collected at the time of postoperative lung function nor the total mean cyclosporine concentration for each patient correlated significantly with the Dco or Kco.

No relationship was found between the total number of weeks with histologic rejection and either the posttransplant Dco or Kco (R = 0.2; p = NS). In addition, there was no correlation between the histologic grade of rejection on the day of lung function testing with any measure of lung function.

Two patients who were CMV-seronegative prior to transplantation received an organ from a CMV-seronegative donor, and both of these patients developed primary CMV infection but no pneumonitis; 11 other patients who were initially CMV-seropositive prior to transplantation also developed active CMV infection posttransplantation (group A, n = 13). Three patients who were CMV-seronegative prior to transplantation remained CMV antibody-negative; there were also six other patients who were CMV-seropositive prior to transplantation but did not develop active CMV infection following transplantation (group B, n = 9). In group B, the two recipients who were CMV-seronegative prior to transplantation received an organ from a seronegative donor. One of these was the only patient of the cohort to develop a Pneumocystis carinii pneumonia, and this patient had the only abnormal chest.

FIGURE 2. Dco pretransplantation and posttransplantation.

FIGURE 3. Kco pretransplantation and posttransplantation.

FIGURE 4. Kco pretransplantation and posttransplantation.
radiograph. No patient in either group had evidence of heart failure or pulmonary emboli at the time of completing the lung function studies. Lung function studies in group A were completed at a mean of 13.6 months following transplant in comparison to a mean of 14.5 months for group B. There was one nonsmoker in group A and two nonsmokers in group B. The mean age of the patients in group A was 52 years and the mean age in group B was 44 years.

In group A, the mean Dco of 4.64 mmol/min/kPa (SD, 0.98) was significantly lower than in group B, 6.16 mmol/min/kPa (SD, 1.06) (p<0.006). In group A, the mean Kco of 1.05 mmol/min/kPa/L (SD, 0.19) was lower than that of group B, 1.2 mmol/min/kPa/L, but this difference was not significant (p = 0.076). The mean percentage reduction in Dco following transplantation was 31 percent in group A and 16.6 percent in group B (p = 0.06) (Fig 1 and 2).

Discussion

A small reduction in lung volumes with relatively preserved gas transfer was present in our patients prior to transplantation. Of the five patients (22 percent) who had reduced preoperative transfer coefficient, two had been taking amiodarone, which is known to cause interstitial fibrosis. Previous data suggest up to one third of patients awaiting heart transplantation have a diffusion defect as the sole abnormality, and there is an inverse relationship between vital capacity and PCWP. Our data suggest that reductions in lung volume are the more usual features of severe congestive cardiac failure prior to surgery. This would be expected to improve following transplantation since the enlarged heart contributes significantly to the loss of lung volume.

The effects of sternotomy and cardiopulmonary bypass on lung function parameters must be considered for several months following surgery. This is unlikely to be important for the majority of our patients as the lung function tests were measured at an average of 13.6 months after transplantation in group A and 14.5 months in group B. The fall in transfer coefficient in our patients suggests that the lung injury is due to factors other than the surgery and cardiopulmonary bypass.

The minor reduction in spirometry combined with the reduction in diffusion capacity could suggest an interstitial process due to cell injury. Interstitial lung disease at a cellular level is associated with endothelial cell injury due to activation of xanthine oxidase by cytokines, including tumor necrosis factor-α (TNF-α). Tumor necrosis factor-α is also recognized as important in the setting of solid organ rejection. However, we found no correlation between severity of rejection at the time of lung function testing and the degree of impairment in diffusion, nor was there any relation to chronicity of rejection.

Cyclosporine has been suggested as being toxic toward the lungs in a similar manner to its effects on the kidney. This suggestion has been reinforced more recently by Groen et al. Cyclosporine could also result in perfusion injury to the lung as a result of microthromboembolus. However, neither the mean whole blood cyclosporine concentration for each patient nor the cyclosporine concentration at the time of lung function correlated with the reduced diffusion capacity. Similarly the difference between the PAP and PCWP following transplantation (PAP-PCWP) did not correlate with the reduced Dco or Kco, suggesting microthromboembolus does not play a role. Vascular injury prior to transplantation does not seem causally linked to the reduction in Dco and Kco following transplantation, since there was no correlation between the pretransplant PVR and the postoperative Dco/Kco.

Cytomegalovirus is an important pathogen in the setting of an immunocompromised host. The risk of development of CMV disease is related to the serologic status of donor and recipient. Up to 90 percent of CMV-seronegative heart and heart lung transplant patients who receive an organ from a CMV-seropositive donor develop CMV disease, but CMV disease may be as low as 30 percent when donor and recipient matching is attempted. Whether serologic evidence of active CMV infection in patients who were CMV seropositive prior to transplantation and who received an organ from a seropositive donor represents reactivation of the patients own virus or reinfection by virus acquired from the donor is unclear. The clinical importance of CMV infection has been emphasized by the observation that it has an important role in rejection and the late development of graft atherosclerosis and graft loss.

We observed a significant difference (p<0.006) between the reduction in diffusion capacity of those transplant patients who had serologic evidence of CMV infection compared to those with no serologic evidence of active infection. Similar findings have been observed in renal transplant recipients with a reduction in diffusing capacity in asymptomatic patients with CMV infection despite a normal chest radiograph. This implies that there may be occult lung injury in the presence of CMV infection in our patients. A diffusion defect at least 3 months after surgery has been described in a different group of clinically well renal transplant recipients, although the presence of CMV infection was not documented in this group. Our observations and those of other groups suggest that occult lung injury is a consistent phenomenon in solid organ transplant recipients.

The common factors to these groups of patients are immunosuppression and infection. The pulmonary dysfunction described in the renal transplant recipi-
ents with CMV infection has been related to complement activation. Lung injury at a cellular level is believed to be due to endothelial cell injury as a result of xanthine oxidase activation by mediators such as TNF-α. The immunologic disturbance in CMV infection is itself based at the macrophage level. It is possible that as a result of immunologic disturbance following transplantation, the release of chemotactic peptides from macrophages such as TNF-α leads to endothelial cell injury in any patient (group B) but this being particularly marked in the setting of CMV infection (group A). Therefore, the diffusion defect provides indirect evidence of ongoing infectious/immune injury that might ultimately contribute to loss of the graft.

We conclude that diffusion impairment is a consistent feature of lung function measurements following heart transplantation and is unrelated to sternotomy, drug toxicity, hemodynamic change, or rejection, but is most pronounced in patients developing CMV infection. A prospective study is required to confirm the relationship between abnormal gas transfer and CMV infection and to investigate its immunopathologic basis.

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