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What is This?
Post-transplantation Lymphoproliferative Disorder in Heart and Kidney Transplant Patients: A Single-Center Experience

Sanjeev Wasson, MD, Mohammad N. Zafar, MD, John Best, MD, and Hanumanth K. Reddy, MD, FACC

Background: Post-transplantation lymphoproliferative disorder (PTLD) after heart transplantation is a fatal complication, and standard treatment is either ineffective or too toxic. We have studied the incidence, clinical course, prognostic factors, and different treatment regimens pertaining to PTLD in 110 heart and 80 kidney transplant recipients.

Methods: Information was abstracted from chart review of 110 heart transplant recipients and 80 kidney transplant recipients between January 1989 and October 2002. We report 15 patients with PTLD, 6 patients received a heart transplant and 9 patients received a renal transplant.

Results: The overall incidence of PTLD was 8.9% (5.4% in heart and 13.7% in kidney transplant recipients). The average interval between transplantation and the diagnosis of PTLD in heart transplantation patients was 5.5 years, and their overall mean age was 44 years. The indications for transplantation were ischemic cardiomyopathy in 5 patients (1 patient received both heart and kidney transplants), glomerulonephritis in 6 patients, diabetes nephropathy in 2 patients, and polycystic disease in 2 patients. Six patients were diagnosed with early disease (<12 months), 7 with late onset (1 to 10 years), and 2 with very late onset (>10 years). Five patients had PTLD grade 2 (2 heart and 3 kidney transplants) and 10 patients had PTLD grade 3 (4 heart and 6 kidney transplants). Immunosuppressive treatment for PTLD patients consisted of cyclosporine, 73% (11/15); tacrolimus, 6.6% (1/15); prednisone, 100% (15/15); azathioprine, 80% (12/15); mycophenolate mofetil, 20% (3/15); murine monoclonal anti-human CD3 (OKT3), 7% (1/15); and anti-thymocyte globulin, 13% (2/15). PTLD developed in 11.5% of patients with primary Epstein-Barr virus infection and in 28.9% of patients with primary cytomegalovirus infection. Five patients received rituximab therapy, 5 had conventional chemotherapy, 3 had radiotherapy, 3 had reduction in immunosuppression, 2 had ganciclovir, 1 underwent surgery, and 1 patient died before receiving treatment. The mortality rate was 26.6%. The average interval between transplantation and the diagnosis of PTLD in heart transplant recipients was 5.5 years. The mortality rate was significantly higher in the control group than in the rituximab group.

Conclusions: Caucasian race and male gender were independent risk factors for developing PTLD. Pretransplant cytomegalovirus seropositive status is a strong predictor of developing PTLD. Management of PTLD requires randomized controlled trials of various chemotherapeutic and antiviral drugs regimens. Treatment of PTLD with rituximab is a beneficial alternative with a favorable outcome. Patients in whom primary Epstein-Barr virus, cytomegalovirus, or hepatitis C infection develop after transplantation should be managed with heightened surveillance for the development of PTLD. Further randomized trials are needed to evaluate the efficacy of antiviral drugs, intravenous immunoglobulin, interferon, and prophylactic Epstein-Barr virus immunization strategies.

Key words: anti-CD20 monoclonal antibody, heart transplant, renal transplant, post-transplantation lymphoproliferative disorder, rituximab.

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Post-transplantation lymphoproliferative disorders (PTLD) represent a spectrum of diseases ranging from a mononucleosis-like illness to lymphoid proliferations or lymphomas that develop in recipients of solid-organ or bone marrow allograft (1,2). PTLD after heart or kidney transplantation is a fatal complication,
and standard treatment is either ineffective or too toxic. Primary Epstein-Barr virus (EBV) infection after transplantation is associated with an increased risk of having PTLD (3). Histologically, PTLD could be polymorphic or monomorphic on microscopic appearance, or polyclonal or monoclonal on molecular analysis. The World Health Organization has classified PTLD into four main categories (Table 1) (4).

Our objective in conducting this study was to determine the incidence, predisposing and prognostic factors, clinical presentation, outcome, and different treatment regimens pertaining to PTLD among patients who receive a heart or kidney transplant, or both.

**Materials and Methods**

**Study Design**

A retrospective analysis to identify all cases of PTLD complicating heart and renal transplantation at the University of Missouri-Columbia (MU) was undertaken. All patients who underwent heart or kidney transplantation at MU between January 1989 and October 2002 were included in this study. Exempt-Waiver was obtained from the MU Institutional Review Board. Information was abstracted from medical chart review of 110 heart transplant recipients and 80 kidney transplant recipients regarding comorbid conditions; EBV, cytomegalovirus (CMV), and hepatitis C serologic findings before and after transplantation; immunosuppression regimens, and the prevalence and treatment of rejection episodes. Charts for those patients who developed PTLD were further reviewed to examine the diagnostic evaluation, treatment, and outcome of PTLD, including the clinical presentation of PTLD and concurrent infections.

**Patient Population**

Study population consisted of 190 patients who underwent organ transplantation between January 1989 and October 2002, of whom 110 (57%) had heart transplantation and 80 (42%) had kidney transplantation. There were 101 men and 89 women. The mean age at the time of transplantation was 44 years (range, 34 to 67 years). The mean age at the time of transplantation was 51 years (median, 51.5 years) for heart and 47.6 years (median, 42 years) for kidney recipients. The average interval between the time of transplantation and the diagnosis of PTLD in heart transplantation patients was 66 months (range, 7 months to 24 years; median, 60 months). Table 2 outlines the characteristics of patients who developed PTLD early, late, or very late. Follow-up of the kidney transplant recipients averaged 53.5 months (range, 2 months to 26 years; median, 65 months).

<table>
<thead>
<tr>
<th>Category</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early lesions</td>
<td>Polyclonal&lt;br&gt;Reactive plasmacytic hyperplasia and an infectious mononucleosis–like presentation&lt;br&gt;Partial architectural preservation of the involved tissue (lymph node or tonsil and adenoids)&lt;br&gt;Younger age&lt;br&gt;Regress spontaneously or after reduction of immunosuppression</td>
</tr>
<tr>
<td>Polymorphic</td>
<td>B-cell maturation&lt;br&gt;Monoclonal&lt;br&gt;Variable response to immunosuppression withdrawal</td>
</tr>
<tr>
<td>Monomorphic</td>
<td>Monoclonal malignant B-cell lymphomas including&lt;br&gt;• diffuse large B-cell lymphoma (immunoblastic, centroblastiic, and anaplastic)&lt;br&gt;• Burkitt or Burkitt-like lymphoma&lt;br&gt;• plasma cell myeloma&lt;br&gt;• plasmacytoma-like lesions&lt;br&gt;Rarely, T-cell neoplasms including peripheral T-cell lymphoma</td>
</tr>
</tbody>
</table>

Hodgkin lymphoma and Hodgkin lymphoma–like PTLD Mostly seen in allogeneic bone marrow transplant recipients
Diagnostic Criteria of PTLD

A definitive diagnosis of PTLD was based on biopsy examination of 6-µm-thick sections of formalin-fixed, paraffin-embedded tissue stained with hematoxylin and eosin. In situ hybridization techniques using the EBV encoded RNA probe for EBV messenger RNA were used to identify EBV sequences in the nuclei of lymphoid cells within the lesion (5). A diagnosis of presumed PTLD was based on documentation of EBV seroconversion of a previously seronegative patient or reactivation of immunoglobulin (Ig) M against EBV viral capsid antigen, along with compatible symptoms (fever, fatigue, anorexia, and respiratory compromise) and the detection of pulmonary nodules or extrapulmonary masses, or both, on radiologic evaluation. This diagnosis required the absence of a concurrent identifiable infection.

Statistical Analysis

Categoric data among groups were compared by the Pearson χ² method. If an expected cell value was less than 5, the Fisher exact test was used. P < .05 was considered significant.

Epstein-Barr Virus, Cytomegalovirus, and Hepatitis C Serologic Status

Pretransplantation EBV serologic status was documented in 106 patients (55.7%), of whom 69 were seronegative and 37 were seropositive at transplantation. None of the seropositive patients showed EBV IgM seropositivity at the time of transplantation, indicating that none of them had a recent infection. Donor EBV serologic status was available for 44 patients, of whom 18 had positive status and 26 had negative status. The EBV status of the donor was not available for 146 patients.

Pretransplantation CMV serologic status was documented in all patients (100%), of whom 152 (80%) were seronegative and 38 were seropositive at transplantation.

Pretransplantation hepatitis C serologic status was documented in 76 patients (40%). All the tested patients were seronegative at transplantation. Other potential risk factors evaluated included age, gender, race, and immunosuppression.

Immunosuppression Treatment Regimen

Overall, immunosuppressive treatment consisted of cyclosporine, tacrolimus, prednisone, azathioprine, mycophenolate mofetil, murine monoclonal anti-human CD3 antibody (OKT3), and antithymocyte gammaglobulin (ATGAM). One hundred twenty-eight patients were treated with an immunosuppressive regimen consisting of cyclosporine, azathioprine, and prednisone. Of these, 68 patients were treated with an immunosuppressive regimen consisting of cyclosporine, azathioprine, and prednisone. Of these, 68 patients were treated with induction therapy using ATGAM or OKT3 (Orthoclone, Ortho Biotech, Bridgewater, NJ) at the time of transplantation. Tacrolimus (formerly known as FK 506) was used as primary immunosuppression in 34 patients. Oral corticosteroid therapy (prednisone, 1 to 2 mg/kg per day) was started in the postoperative period and usually had been tapered or discontinued by 3 to 6 months after transplantation.

Of the 15 patients who developed PTLD, 11 received cyclosporine, 1 received tacrolimus, 1 received OKT3, 2 received ATGAM, 3 received mycophenolate, and 12 received azathioprine. All 15 received prednisone. The detailed immunosuppressive regimen and PTLD treatment is summarized in Table 3.

Target levels for either tacrolimus or cyclosporine were empirically set on an individual basis according to a variety of factors that included the organ transplanted, rejection history, age, renal and hepatic function, and nutritional status. In general, target whole blood trough concentrations of tacrolimus immediately after kidney transplantation were 5 to 15 ng/mL, with lower target levels (5 to 12 ng/mL) after 6 to 12 months post-transplant. Target tacrolimus levels (whole blood levels) for maintenance immunosuppression in heart transplant recipients

<table>
<thead>
<tr>
<th>Population Characteristics and Mean Time Interval to Development of Post-transplantation Lymphoproliferative Disorder</th>
<th>Early (&lt;1 year)</th>
<th>Late (1 to 10 years)</th>
<th>Very Late (&gt;10 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age</td>
<td>44</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Gender (Male)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients developing PTLD early, late, and very late after transplant</td>
<td>6</td>
<td>7</td>
<td>2</td>
</tr>
</tbody>
</table>
Plasma or whole blood cyclosporine trough levels were routinely monitored. The target maintenance cyclosporine levels were 300 to 700 ng/mL (whole blood radioimmunoassay) for all heart transplant recipients. No dosage adjustments were made unless the kidney transplant recipient had levels that were persistently elevated (>150 ng/mL in the plasma or >300 ng/mL in whole blood) or reduced (<50 ng/mL in plasma or <150 ng/mL in whole blood) in association with a rising plasma creatinine concentration.

Two-hour peak cyclosporine or C2 levels targets were 1000 to 1500 ng/mL in the first 6 months after kidney transplantation and 800 to 900 ng/mL in months 6 to 12 and 500 to 800 ng/mL for subsequent months.

Augmented immunosuppression was used in the treatment of acute rejection episodes in 122 patients. This included corticosteroid pulse therapy, ATGAM, or OKT3, usually with an increased maintenance cyclosporine or tacrolimus dosage.

**Results**

We report 15 patients with PTLD: 6 (5.4%) of 110 heart transplant recipients and 9 (11.2%) of 80 renal transplant recipients. The higher occurrence of PTLD in renal transplant recipients did not reach statistical significance compared with heart transplant recipients ($P = .28$). The overall incidence of PTLD was 8.9% in heart and kidney transplantation patients when considered as one group.

Eleven were men, four were women ($P = .172$). Thirteen patients were white, one woman was African-American, and one man was African-American ($P = .102$). The mean age at the time of transplantation was 44 years. Caucasian race and male gender were independent risk factors with odds ratios (OR) of 2.423 and 2.597, respectively ($P = .04$). The combination of all three risk factors increased the OR to 2.66.

Young white men are at highest risk for PTLD development among recipients of solid-organ transplants.

The indications for transplantation were ischemic cardiomyopathy in 5 patients (1 patient received a heart and kidney transplant), glomerulonephritis in 6 patients, diabetes nephropathy in 2 patients, and polycystic disease in 2 patients (Table 4).

PTLD developed in 8 (11.5%) of 69 previously EBV-seronegative patients and in 1 EBV-seronegative

<table>
<thead>
<tr>
<th>Patient</th>
<th>Immunosuppressive Regimen</th>
<th>Treatment Regimen for PTLD/ (Duration of Treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cyclosporine, 175 mg bid; prednisone (tapered); azathioprine, 50 QD</td>
<td>Rituximab (1 cycle)</td>
</tr>
<tr>
<td>2</td>
<td>Cyclosporine, prednisone, azathioprine</td>
<td>CHOP (3 cycles), Rituximab (1 cycle)</td>
</tr>
<tr>
<td>3</td>
<td>Tacrolimus, 7 mg qd; mycophenolate mofetil 100 mg bid (later: FK 506)</td>
<td>Discontinuation of immunosuppression, Rituximab (1 cycle), ganciclovir</td>
</tr>
<tr>
<td>4</td>
<td>Cyclosporine, 175 mg bid; Prednisone, 10 mg QD; azathioprine, 75 mg bid</td>
<td>Supportive</td>
</tr>
<tr>
<td>5</td>
<td>Azathioprine, prednisone, cyclosporine, ATGAM</td>
<td>CHOP (1 cycle)</td>
</tr>
<tr>
<td>6</td>
<td>Cyclosporine, 350 mg bid; azathioprine, 125 mg QD; prednisone, 10 mg QD</td>
<td>Ganciclovir</td>
</tr>
<tr>
<td>7</td>
<td>Cyclosporine 400 mg bid; azathioprine,150 mg QD; prednisone, 25 mg QD</td>
<td>Decreased immunosuppression</td>
</tr>
<tr>
<td>8</td>
<td>Azathioprine, high-dose prednisone</td>
<td>Discontinuation of immunosuppression, CHOP (1 cycle)</td>
</tr>
<tr>
<td>9</td>
<td>Cyclosporine 500 mg bid; azathioprine, 150 mg QS; prednisone, 75 mg QD</td>
<td>CHOP (2 cycles), radiation</td>
</tr>
<tr>
<td>10</td>
<td>Cyclosporine, 325 mg bid; azathioprine, 50 mg; prednisone, 70 mg QD after renal transplant</td>
<td>Surgery, radiation (no evidence of recurrence)</td>
</tr>
<tr>
<td>11</td>
<td>Cyclosporine, 325 bid; azathioprine, 100 bid; prednisone, 50 bid</td>
<td>Diagnosed at autopsy</td>
</tr>
<tr>
<td>12</td>
<td>Cyclosporine, prednisone (OKT-3 for acute rejection)</td>
<td>Radiation, CVP x3 cycles</td>
</tr>
<tr>
<td>13</td>
<td>Cyclosporine, mycophenolate mofetil, prednisone</td>
<td>Decreased immunosuppression, rituximab</td>
</tr>
<tr>
<td>14</td>
<td>Azathioprine, ATGAM, prednisone, mycophenolate mofetil</td>
<td>Cytarabine, ifosfamide, mesna, methotrexate</td>
</tr>
<tr>
<td>15</td>
<td>Cyclosporine, azathioprine, prednisone</td>
<td>CHOP (3 cycles), Rituximab (2 cycles)</td>
</tr>
</tbody>
</table>

CHOP = chemotherapy; CVP = cyclophosphamide, vincristine and prednisone.
A patient who remained seronegative after transplantation. EBV status before transplantation was not documented in 6 patients in whom PTLD developed. None of the 37 seropositive patients for EBV before transplantation developed PTLD. This points to a significant association between the occurrence of PTLD and development of primary EBV infection after transplantation compared with patients seropositive for EBV before transplantation (\(P < .028\)).

CMV serologic status before transplantation was documented in all patients (100%), of whom 152 (80%) were seronegative and 38 were seropositive at transplantation. PTLD developed in 11 (28.9%) of 38 seropositive patients for CMV and in 4 (2.6%) of 152 seronegative patients for CMV (\(P = .001\)), indicating that CMV is a strong predictor for the development of PTLD.

Hepatitis C serologic status before transplantation was documented in 76 patients (40%). All the tested patients were seronegative at transplantation and 19.7% of HCV-negative patients developed PTLD, which failed to reach any statistical significance (\(P = .001\)). Their lactic dehydrogenase levels at the time of transplantation averaged at 256.4 U/L.

### Clinical Presentation and Diagnosis of PTLD

Table 5 summarizes the varied clinical presentation and pathologic biopsy results of all the patients diagnosed with PTLD. EBV was present in all the biopsy specimens tested by in situ hybridization. Monomorphic histology was present in 9 of the 15
specimens. The average interval between transplantation and the diagnosis of PTLD in heart transplantation patients was 5.5 years. Six patients were diagnosed with early disease (<12 months), 7 with late onset (1 to 10 years) and 2 with very late onset (>10 years). Five patients had PTLD grade 2 (2 heart and 3 kidney transplants) and 10 patients had PTLD grade 3 (4 heart and 6 kidney transplants).

Immunosuppression Treatment Regimen

Immunosuppressive treatment of PTLD patients consisted of cyclosporine, 73% (11/15), tacrolimus, 6.6% (1/15); prednisone, 100% (15/15); azathioprine, 80% (12/15); mycophenolate mofetil, 20% (3/15); OKT3, 7% (1/15); and ATGAM, 13% (2/15). Analysis of immunosuppressive therapy (either the primary immunosuppression regimen or the augmented immunosuppressive protocol used for induction or treatment of rejection episodes) revealed a significant correlation of degree of immunosuppression with the development of PTLD ($P = .029$).

Treatment and Patient Outcomes

Therapy for PTLD consisted of standard doses of rituximab therapy over 4 weeks ($n = 5$), conventional chemotherapy ($n = 5$), radiotherapy ($n = 5$), decrease in immunosuppression ($n = 3$), antiviral therapy consisting of ganciclovir ($n = 2$), and surgery ($n = 1$). Of the 15 patients with PTLD, 1 patient died before receiving treatment, PTLD contributed to death of 3 patients (2 heart and 1 kidney transplant recipients), and 11 patients were alive 11 months to 5.9 years after the diagnosis of PTLD. The mortality rate was 26.6%.

On further analysis of PTLD patients among heart transplant recipients, we divided these patients into two groups based on their treatment: one group received conventional treatment, and the other received rituximab. The incidence of EBV in the control group was 75% (3/4), and in the rituximab group it was 0% (0/2). Three (75%) of 4 patients were CMV positive in the control group and 1 (50%) of 2 in the rituximab group. The immunosuppressive protocol was identical in both groups and consisted of cyclosporine, prednisone, and azathioprine. Only one patient in the conventional group received OKT3. None of these patients received ganciclovir. The mortality rate in the control group was 50% (2/4) and 0% in the rituximab group ($P < .001$).

Discussion

Solid-organ transplantation carries a significant risk of PTLD. In our study population, PTLD was diagnosed in 8.9% of transplant recipients, with 5.4% of heart and 11.2% of renal transplant recipients. This contrasts with a 3.4% incidence in adult heart and 1.0% in adult kidney transplantation patients at the University of Pittsburgh (6). Our group acknowledges that this small cohort cannot be used to draw generalized conclusions. We do, however, report a higher incidence of
PTLD than previously published and the need for further randomized trials based on trends at our institute.

**Pathophysiology**

Risk factors for the development of PTLD for solid-organ transplantation patients include an EBV seronegative recipient status, development of a primary EBV infection or, occasionally, reactivation of EBV after transplantation; presence of CMV disease or hepatitis C, younger recipient age, white race, male gender, and finally, the degree of immunosuppression.

The available data suggest that the primary EBV infection after transplantation is a stronger risk factor for the development of PTLD than the reactivation of recipient latent EBV infection (7). In addition to primary EBV infection after transplantation, CMV infection is the major risk factor associated with the development of PTLD, which is in agreement with the earlier published reports for heart transplant recipients (3). Lymphotropic stimulation of the immune system by CMV could explain its association with the development of PTLD.

The type of transplanted organ and lactic dehydrogenase levels did not significantly influence the survival. The interval between transplantation and PTLD diagnosis (>1 year) showed a trend towards statistical significance, with an interval of more than 1 year being associated with poor survival. Armitage (3) also found that the mortality rate in late-onset PTLD was higher than in early-onset PTLD, and that late-onset PTLD was often disseminated.

**Immunosuppressive Drug Treatment**

The treatment for PTLD is mainly based on the clinical outcomes described in case reports or a limited series of patients. Various anecdotal reports have described the use of antiviral therapy (acyclovir or ganciclovir), immunoglobulin preparations, interferon α-2b therapy, monoclonal anti-B-cell antibodies against CD21 and CD24, and cell therapy. An anti-CD20 antibody (rituximab) preparation used in a small group of patients with refractory PTLD showed better survival compared with conventional chemotherapy (8).

**Prevention**

Transplant recipients who are at high risk for PTLD should be closely followed with physical examinations, chest radiographs, computed tomography scans of the head, thorax, and abdomen; and monitoring of EBV, CMV, and hepatitis C viral loads. EBV, CMV, and hepatitis C serostatus should be determined for all potential transplant recipients. Physicians taking care of transplantation patients should have a higher suspicion of PTLD in such patients with EBV, CMV, or hepatitis C infection, lymphadenopathy, abdominal mass, head and neck masses, pulmonary nodules, or skin lesions. Early recognition is crucial in the management of PTLD. Further randomized trials need to be conducted to evaluate the efficacy of various newer therapies, including prophylactic antiviral drugs such as ganciclovir for CMV, intravenous immunoglobulin, interferon, and preventive strategies such as EBV vaccines.

**Conclusions**

PTLD is potentially a fatal condition in posttransplant population. Treatment of PTLD is difficult and challenging. The risk of a patient developing PTLD seems to be related to EBV, CMV, or hepatitis C status, age, race, gender, and finally, the degree of immunosuppression. The higher incidence of PTLD in transplantation patients that we have reported points to a need for randomized controlled trials of various chemotherapeutic and antiviral drugs regimens. Long-term prognosis can be improved with early recognition and appropriate therapy.

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