Posttransplant lymphoma in renal and heart allograft recipients: a single center experience

Ayşen TİMURAĞAOĞLU[1], Aynur UĞUR BİLGİN[1], Murat TUNCER[2], İlhan GÖLBAŞI[3], Dilek ÇOLAK[4], Seray DİZLEK[5], Gülten KARPUZOĞLU[6]

[1] Department of Haematology, Akdeniz University School of Medicine,
[2] Department of Nephrology, Akdeniz University School of Medicine,
[3] Department of Cardiovascular Surgery, Akdeniz University School of Medicine,
[4] Department of Microbiology and Clinical Microbiology, Akdeniz University School of Medicine,
[5] Molecular Haematology Unit of Central Laboratory, Akdeniz University School of Medicine,
[6] Department of Pathology, Akdeniz University School of Medicine, Antalya, TURKEY

Turk J Haematol 2005;22(4): 197-203

Received: *29.02.2005* ***Accepted:*** *21.07.2005*

ABSTRACT

Posttransplant lymphoproliferative disorder (PTLD) is a serious complication of organ transplantation, with a reported incidence between 0.8% to 32%. Herein we retrospectively analyzed the patients who diagnosed as PTLD in Akdeniz University. Within the 782 (773 renal and 9 heart) transplant recipients six patients were diagnosed as PTLD (diffuse large B-cell lymphoma). Five of them had renal, one had cardiac transplantation. Three patients were diagnosed within the first year of transplantation. Five patients had abdominal disease one had central nervous system involvement. All patients had positive Epstein-Barr virus (EBV) and cytomegalovirus (CMV) IgG at the time of diagnose. EBV-DNA with polymerase chain reaction (PCR) was found to be negative in five patients. Only one patient was survived after the diagnosis of PTLD. In conclusion, even with treatment the mortality rate is high in patients with PTLD. In order to decrease the incidence of PTLD and related mortality, the risk factors should be evaluated with multicenter studies.

Key Words: PTLD, EBV, CMV, Renal transplantation, Heart transplantation.

ÖZET

Böbrek ve kalp allograft alıcılarında posttransplant lenfoma: Tek merkez deneyimi

Transplantasyon sonrası gelişen lenfoproliferatif hastalık (PTLH) organ nakillerinin önemli bir komplikasyonu olup, %0.8 ile %32 oranında görülmektedir. Burada Akdeniz Üniversitesi'nde tespit edilen PTLH olgularını retrospektif olarak değerlendirmeyi amaçladık. Transplantasyon yapılmış olan 782 (773 böbrek, 9 kalp) hastanın altısında difüz büyük B-hücreli lenfoma gözlenmiştir. Olguların beşi böbrek, biri kalp nakli yapılmış hastalar olup, üç hastada ilk bir yıl içinde lenfoma geliştiği tespit edilmiştir. Olguların beşinde hastalık batından, birinde santral sinir sisteminden kaynaklanmıştır. Tüm hastalarda lenfoma tanısı aldıkları sırada Epstein-Barr virüs (EBV)

ve sitomegalovirüs (CMV) IgG pozitif, beş olguda polimeraz zincir reaksiyonu ile EBV-DNA negatif bulunmuştur. Tanıdan sonra yapılan tedaviler ile yalnızca bir olgu hayatta kalabilmiştir. Sonuç olarak, tedaviye rağmen mortalitesi yüksek olan PTLH sıklığını ve buna bağlı ölüm oranlarını azaltabilmek için risk faktörlerinin çok merkezli çalışmalarla tespit edilmesi gerekmektedir.

Anahtar Kelimeler: PTLH, EBV, CMV, Böbrek transplantasyonu, Kalp transplantasyonu.

INTRODUCTION

Posttransplant lymphoproliferative disorder (PTLD) is a well-known complication of solid organ transplantation and it is an important cause of morbidity and mortality in transplant recipients. Typically, PTLD represents an uncontrolled proliferation of lymphoid cells from benign polyclonal lymphoid hyperplasia to aggressive monoclonal lymphoma[1,2].

Many probable risk factors have been identified for PTLD up to date. The type of graft, kind and intensity of immunosuppressive drugs, age of recipient, infectious agents particularly viruses [Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis C virus (HCV)], are some of the most important reported risk factors of this disorder[3-8]. Depending on the patients age groups and the type of allograft the incidence of PTLD varies between 0.8% to 32%. The highest rate was found in children with small-intestine transplants[9]. Herein we retrospectively analyzed the patients who diagnosed as PTLD in Akdeniz University.

MATERIALS and METHODS

Between January 1993 and July 2004, 782 (773 renal and 9 heart) patients underwent transplantation at Akdeniz University. The patients with PTLD were identified and their medical records were reviewed. Gender, sex, clinical presentation, immunosuppressive therapy, tumor characteristics and clinical outcome were evaluated.

For immunosuppression after transplantation, cadaveric organ recipients (220 patients) received polyclonal antithymocyte globulin (ATG 2.5 mg/kg/day for 7-14 days) as an induction therapy. As a maintenance therapy, cadaveric and living-related recipients got prednisolone (PRD), azathioprine (AZT) (100 mg/first day), mycophenolate mophetil (MMF) (2 g/per day) and cyclosporine A (CsA) (8 mg/per day) or tacrolimus (FK 506) (0.15 mg/first day). CsA and tacrolimus dosages were modulated according to blood levels. All patients received acyclovir (3.2 g/day according to glomeruler filtration rate) therapy for six months after transplantation.

Patients were ordinarily seen in the outpatient clinic for two years. At each visit, physical examination, biochemical tests and whole blood counts, blood levels of CsA or FK 506 were determined. In addition the incidence of acute rejection episodes were recorded in every case.

The HCV, CMV and EBV serology of cardiac and renal transplant recipients were reviewed and we were able to reach most of them but we couldn't obtain viral serology of cadaveric donors except human immunodeficiency virus (HIV).

The pathologic tissue specimens of patients were reevaluated and additionally if there was enough tissue immunohistochemistry for latent membrane protein (LMP) and immunophenotyping for LCA, CD3 and CD20 were performed. DNA was isolated from paraffin embedded tissue and EBV-DNA was searched with polymerase chain reaction (PCR) (EBV-Copies, quantitative determination of EBV-DNA, ARGENE-BİOSOFT, Varilhes, France).

RESULTS

In this study we identified six patients (five male and one female) with PTLD. Their median age was 40, five year (range 25-61 years). The clinical characteristics of patients were given on Table 1. Five of them had renal (0.6%), one had cardiac transplantation (11%). Two patients were transplanted cadaveric, four patients living related donor organ. The median duration from transplantation to diagnosis of lymphoma was 52 months (range 2-101 months). PTLD was diagnosed in three patients within the first year of transplantation. All of the patients with PTLD had positive EBV and CMV serology (IgG) before transplantation and just at the time of lymphoma diagnosis. Three patients developed positive CMV antigenemia after the transplantation. The HCV antibody was found to be negative in all PTLD patients.

The anti-HCV, EBV IgM and G, CMV IgM and G of transplanted patients with or without PTLD and donors were given on Table 2. CMV IgG and IgM were negative in two recipients and their donor. CMV IgG and M were negative in thirteen recipients but they received organ from IgG positive donors. One recipient was both EBV IgG and M negative but his donor had positive IgM and G antibodies.

Within 782 organ recipients 196 received cadaveric organ.

We obtained only five patients' pathological specimens and reevaluated them. The diagnosis was confirmed with morphological examination and with immunophenotyping studies and they were all identified as diffuse large B-cell lymphoma (CD20 positive). In addition, we searched for EBV-DNA with PCR on the pathologic specimen but we found them negative. We were able to perform immunohistochemistry for EBV LMP for only three specimens and all of them were found to be negative. Characteristics of

Table 1. The characteristics of patients with PTLD

No	Age	Gender	Allograft	Etiology of disease	Donor	Immunosuppressive drugs
1	32	M	Kidney	Polycystic renal disease	LR	PRD + CsA + MMF-AZT
2	25	F	Kidney	U	LR	PRD + CsA + MMF-AZT
3	63	M	Heart	Cardiomyopathy	C	PRD + ATG + CsA + MMF
4	49	M	Kidney	U	C	ATG + PRD + MMF + FK506
5	46	M	Kidney	Chronic glomerulonephritis	LR	AZT + PRD + CsA
6	35	M	Kidney	Familial Mediterranean Fever-Amiloidozis	LR	PRD + AZT

LR: Living related, U: Unknown, C: Cadaveric, PRD: Prednisolone, CsA: Cyclosporine A, MMF: Mycophenolate mophetil, AZT: Azathioprine, FK506: Tacrolimus, ATG: Antithymocyte globulin.

Table 2. The viral serology of recipients, donors and PTLD patients and their donors

	Anti-HCV		EBV IgM		EBV IgG		CMV IgM		CMV IgG	
	+	-	+	-	+	-	+	-	+	-
Recipient	41	540	9	351	321	39	3	412	398	17
Donor	0	395	10	293	275	28	2	258	257	13
PTLD	0	6	0	5	3	2	0	5	5	0
Donor	0	3	0	3	2	1	0	3	3	0

patients according to therapy, involved tissue and complications were given on Table 3.

Five patients were died due to disease progression or chemotherapy complications within six months. Only one patient who diagnosed as PTLD a year ago, is still alive.

DISCUSSION

Between 1973 and 2004 within the 782 allograft recipients six patients developed PTLD in our hospital. The incidence of PTLD was found to be consistent with the other studies which was found higher rates in cardiac transplantation than renal transplantation [11% (1/9) and 0.6% (5/773)][9-13]. The highest rates were reported in T cell depleted bone-marrow and small intestine recipients. Though the immunosuppressive agents that were used nearly the same in all kind of transplantations the different rates probably evolved from the biologic differences of grafts.

It was reported that the donor recipient mismatch of EBV serostatus and seroconversion or primary infection of EBV after transplantation increase the risk of PTLD but EBV negative PTLD was also reported especially within the late occurring PTLD. In our study group, one recipient was both EBV IgG and M negative but his donor had positive IgM and G antibodies but this patient did not progress to PTLD. We had three early, three late occurring PTLD but, we could not able to detect EBV-DNA in the involved tissue of five of them with PCR. We used a kit for PCR which detects a fragment located in BNRF1 of EBV genome. EBV latent gene and antigen expression are different in different malignancies. For example African Burkitt's lymphoma is lack of LMP antigen[14]. We don't know if BNRF1 fragment of EBV-DNA is found in PTLD but we know that LMP is found in most of them. EBV LMP was studied by immunohistochemistry only two early onset and one late onset PTLD and all of them were found to be negative. EBV encoded RNA (EBER) with in situ hybridization and EBV-DNA with PCR are both powerful detection methods of EBV in tissues. We couldn't perform EBER because of technical reasons and we studied EBV-DNA from paraffin embedded tissue. Isolated DNA was controlled in all tissues with a part of human genome but if there was sparse infiltration we may have not obtained involved tissue with lymphoma and so we could have not shown EBV-DNA. It was also reported that EBV-DNA was not amplify well from DNA which extracted paraffin embedded tissues

Table 3. The characteristics of PTLD patients according to therapy, involved tissue and complications

No	Duration between transplantation and lymphoma (months)	Site of lymphoma	Treatment	Survive	Cause of death
1	92	Stomach, liver	CHOP	Alive	
2	77	Abdominal mass	CHOP	Dead	Pneumonia + gastrointestinal bleeding
3	10	Retroperitoneal mass, pleural effusion	CHOP	Dead	Sepsis
4	2	Spleen	CHOP	Dead	Hemophagocytic syndrome
5	12	Small bowel	CHOP	Dead	Sepsis
6	94	Cerebrum		Dead	Intracranial bleeding

CHOP; C: Cyclophosphamide, H: docsorubicin HCL, O: Vincristine, P: Prednisolone.

and they try to optimize the reaction[15]. And lastly probably whole EBV genome does not integrated in to cellular components and so we could not amplify it. It is not possible to comment that all of the patients were EBV negative but there were nothing to implicate its association in our patients.

CMV association with PTLD has also been reported and it was shown that CMV itself has an immunosuppressive effect. Together with immunosuppressive agents CMV increases the risk of PTLD[4]. Three of our patients had positive CMV antigenemia. Probably CMV was the most important causative agent in here but with only positive antigenemia it is probably obstacle to implicate their association.

The preventive effect of gancyclovir and following with acyclovir has been speculated[16-18]. Our patients with PTLD had taken acyclovir for six months after the transplantation and additionally three of six patients also used gancyclovir for positive CMV antigenemia. According to our study antiviral agents are not preventive in transplant patients.

The immunosuppressive drugs were also accused for early PTLD. In 1969, it was first described as a complication of azathioprine based therapy by Penn et al[19]. Many modifications in posttransplant management have been made over the last years. These include OKT3, ATG, MMF and FK506[20]. Although the number of rejection episodes with the new agents was decreased, the incidence of PTLD did not change. All patients in this study had received STR + AZT or MMF + CsA or FK506 as a maintenance therapy. Additionally two patients received ATG also. Recently, Birkeland et al could not show the increased risk in any drug regime in the development of PTLD[21]. Probably the immunosuppressive agents did not increase the risk in our patient group. As a result, because of our study population was too small it was not possible to find out any risk factor. But there may be different factors that increase the risk of PTLD. We can speculate that the regional factors such as environmental factors or polymorphisms of some genes like cytokines, MTHFR, may be different and they may increase the risk of PTLD.

PTLD mostly presents as a systemic disease involving lymphoid tissue, spleen, central nervous system or another extralymphoid region or sometimes allograft[21,23]. None of the patients had palpable lymph node in present study. Three of our patients had gastrointestinal system involvement. Interestingly, we determined spontaneous splenic rupture due to involvement of lymphoma without enlargement of spleen in one of the patients who presented as early PTLD. This patient died with hemophagositic syndrome and disseminated intravascular coagulation. Two patients presented with abdominal discomfort due to a retroperitoneal mass. The other patient had CNS involvement. Although primary CNS lymphoma is relatively rare in general population, it is common in organ recipients and immunosuppressed patients[24-26]. We should suspect CNS lymphoma whenever a transplant patient has neurological symptoms.

Despite numerous advances in this disease, the optimum treatment approach is not completely determined[26,27]. Reduction of immunosuppressive therapy, antiviral drugs, chemotherapy and irradiation are the most important attempts. However, chemotherapy has considerable toxicities especially myelosuppression. Swinnen et al reported sepsis and cardiac toxicity depending chemotherapy drugs in their cardiac recipient[28]. In this report, three of six PTLD patients died with infection (sepsis and pneumonia) as a complication of chemotherapy.

In conclusion, PTLD is one of the most important complications of organ transplantation and even with treatment, the mortality rate is high. In order to decrease PTLD and mortality the risk factors should be evaluated with multicenter studies.

REFERENCES

1. Yousem SA, Randhawa P, Locker J, Paradis IL, Dauber JA, Griffith BP, Nalesnik MA. Posttransplant lymphoproliferative disorders in heart-lung transplant recipients: primary presentation in the allograft. Hum Pathol 1989;20:361-9.
2. Nalesnik MA, Makowka L, Starzl TE. The diagnosis and treatment of posttransplant lymphoproliferative disorders. Curr Probl Surg 1988;25:367-472.
3. Walker RC, Marshall WF, Strickler JG, Wiesner RH, Velosa JA, Habermann TM, McGregor CG, Paya CV. Pretransplantation assessment of the risk of lymphoproliferative disorder. Clin Infect Dis 1995;20: 1346-53.
4. Manez R, Breinig MC, Linden P, Wilson J, Torre-Cisneros J, Kusne S, Dummer S, Ho M. Posttransplant lymphoproliferative disease in primary Epstein-Barr virus infection after liver transplantation: the role of cytomegalovirus disease. J Infect Dis 1997;176:1462-7.
5. Cox KL, Lawrence-Miyasaki LS, Garcia-Kennedy R, Lennette ET, Martinez OM, Krams SM, Berquist WE, So SK, Esquivel CO. An increased incidence of Epstein-Barr virus infection and lymphoproliferative disorder in young children on FK506 after liver transplantation. Transplantation 1995;59:524-9.
6. Beveridge T, Krupp P, McKibbin C. Lymphomas and lymphoproliferative lesions developing under cyclosporin therapy. Lancet 1984;1:788.
7. Swinnen LJ, Costanzo-Nordin MR, Fisher SG, O'Sullivan EJ, Johnson MR, Heroux AL, Dizikes GJ, Pifarre R, Fisher RI. Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. N Engl J Med 1990;323:1723-8.
8. Keay S, Oldach D, Wiland A, Klassen D, Schweitzer E, Abruzzo LV, Kumar D, Bartlett S. Posttransplantation lymphoproliferative disorder associated with OKT3 and decreased antiviral prophylaxis in pancreas transplant recipients. Clin Infect Dis 1998; 26:596-600.
9. Finn L, Reyes J, Bueno J, Yunis E. Epstein-Barr virus infections in children after transplantation of the small intestine. Am J Surg Pathol 1998;22: 299-309.
10. Mihalov ML, Gattuso P, Abraham K, Holmes EW, Reddy V. Incidence of post-transplant malignancy among 674 solid-organ-transplant recipients at a single center. Clin Transplant 1996;10:248-55.
11. Hanto DW, Frizzera G, Gajl-Peczalska KJ, Simmons RL. Epstein-Barr virus, immunodeficiency, and B cell lymphoproliferation. Transplantation 1985;39: 461-72.
12. Boubenider S, Hiesse C, Goupy C, Kriaa F, Marchand S, Charpentier B. Incidence and consequences of posttransplantation lymphoproliferative disorders. J Nephrol 1997;10:136-45.
13. Ellis D, Jaffe R, Green M, Janosky JJ, Lombardozzi-Lane S, Shapiro R, Scantlebury V, Vivas C, Jordan ML. Epstein-Barr virus-related disorders in children undergoing renal transplantation with tacrolimus-based immunosuppression. Transplantation 1999; 68:997-1003.
14. Kieff E. Epstein-Barr virus. In: Gorbach SL, Bartlett JG, Blacklow NR (eds). Infectious Disease. 3rd ed. Philadelphia: Lipincott-Williams-Wilkins, 2004:1935-47.
15. Yukio S, Takasaka N, Yoshiko H, Osaki M, Ohfuji S, Ito H, Kaibara N, Kurata T, Sairenji T. Pretreatment with resyriction enzyme or bovine serum albumin for effective PCR amplification of Epstein-Barr Virus DNA in DNA extracted from paraffin-embedded gastric carcinoma tissue. J Clin Microbiol 1998;36: 3423-5.
16. Preiksaitis JK, Diaz-Mitoma F, Mirzayans F, Roberts S, Tyrrell DL. Quantitative oropharyngeal Epstein-Barr virus shedding in renal and cardiac transplant recipients: relationship to immunosuppressive therapy, serologic responses, and the risk of posttransplant lymphoproliferative disorder. J Infect Dis 1992;166:986-94.
17. Davis CL, Harrison KL, McVicar JP, Forg PJ, Bronner MP, Marsh CL. Antiviral prophylaxis and the Epstein Barr virus-related post-transplant lymphoproliferative disorder. Clin Transplant 1995;9:53-9.
18. Darenkov IA, Marcarelli MA, Basadonna GP, Friedman AL, Lorber KM, Howe JG, Crouch J, Bia MJ, Kliger AS, Lorber MI. Reduced incidence of Epstein-Barr virus-associated posttransplant lymphoproliferative disorder using preemptive antiviral therapy. Transplantation 1997;64:848-52.
19. Penn I, Hammond W, Brettschneider L, Starzl TE. Malignant lymphomas in transplantation patients. Transplant Proc 1969;1:106-12.
20. Marker SC, Ascher NL, Kalis JM, Simmons RL, Najarian JS, Balfour HH Jr. Epstein-Barr virus antibody responses and clinical illness in renal transplant recipients. Surgery 1979;85:433-40.
21. Birkeland SA, Hamilton-Dutoit S. Is posttransplant lymphoproliferative disorder (PTLD) caused by any specific immunosuppressive drug or by the transplantation per se? Transplantation 2003;76:984-8.
22. Hanto DW, Gajl-Peczalska KJ, Frizzera G, Arthur DC, Balfour HH Jr, McClain K, Simmons RL, Najarian JS. Epstein-Barr virus (EBV) induced polyclonal and monoclonal B- cell lymphoproliferative diseases occurring after renal transplantation. Clinical, pathologic, and virologic findings and implications for therapy. Ann Surg 1983;198:356-69.
23. Leblond V, Sutton L, Dorent R, Davi F, Bitker MO, Gabarre J, Charlotte F, Ghoussoub JJ, Fourcade C, Fischer A. Lymphoproliferative disorders after organ transplantation: a report of 24 cases observed in a single center. J Clin Oncol 1995;13:961-8.

24. Kalayoglu-Besisik S, Kocaman O, Erer B, Yenerel M, Dogan O, Sargin D. The outcome of large B-cell lymphoma evolving in a hematopoietic stem transplant patient during treatment of chronic graft-versus-host disease. Turk J Haematol 2003;20:2003.
25. Micallef IN, Chhanabhai M, Gascoyne RD, Shepherd JD, Fung HC, Nantel SH, Toze CL, Klingemann HG, Sutherland HJ, Hogge DE, Nevill TJ, Le A, Barnett MJ. Lymphoproliferative disorders following allogeneic bone marrow transplantation: the Vancouver experience. Bone Marrow Transplant 1998;22:981-7.
26. Swinnen LJ. Diagnosis and treatment of transplant-related lymphoma. Ann Oncol 2000;11(Suppl 1):45-8.
27. O'Neill BP, Illig JJ. Primary central nervous system lymphoma. Mayo Clin Proc 1989;64:1005-20.
28. Reams BD, McAdams HP, Howell DN, Steele MP, Davis RD, Palmer SM. Posttransplant lymphoproliferative disorder: incidence, presentation, and response to treatment in lung transplant recipients. Chest 2003;124:1242-9.

Address for Correspondence:

Ayşen TİMURAĞAOĞLU, MD

Department of Haematology,
Akdeniz University School of Medicine
Antalya, TURKEY

e-mail: *aysentimuragaoglu@yahoo.com*