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Post-transplant lymphomas: a 20-year epidemiologic, clinical and pathologic study in a single center

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Background and Objectives. To study the incidence, clinical presentation, pathologic features and outcome of post-transplant lymphomas (PTL) during the past 20 years.

Design and Methods. We undertook a descriptive study of all biopsy-proven cases of PTL diagnosed in our hospital from 1979 through 1999. The average annual incidence rate of PTL was analyzed at 5-year intervals from 1979 to 1999. Risk ratios were estimated by comparing the incidence of PTL among transplanted patients with that of lymphoma observed in the general population of the region. Survival analysis was performed at the univariate level using the Kaplan Meier technique and at the multivariate level by Cox hazard models.

Results. Seventeen of 1,860 transplanted patients developed a PTL (0.9%). The risk of PTL was calculated to be almost 8-fold higher than the risk of lymphoma in the general population. The risk was highest among those who had received a heart transplant (RR=35.6). The mean time between transplant and the diagnosis of PTL was 31 ± 29 months. Of all PTL, 88% were of B-cell origin and 53% of the cases tested were Epstein-Barr virus (EBV)-positive. The median survival was 24 months. The majority of patients with allograft involvement died within the 2 months following diagnosis (hazard ratio 5.3; 95% CI 1.4-20.7).

Interpretation and Conclusions. Organ transplantation is a major risk factor for the development of lymphoma, a disease with a particularly bad prognosis when it develops at the site of the allograft. Early diagnosis and more specific treatment may improve PTL survival.

Key words: post-transplant lymphomas, Epstein-Barr virus, immunosuppression

Lymphomas are one of the most frequent tumors occurring in organ transplant recipients, comprising 20% of all neoplasms.[1,2] They are a serious complication and occur with an incidence that ranges from 0.7% to 4.6%, depending on the degree and duration of immunosuppression used, the organ transplanted, and the age of the transplant recipients.[3-8] The induction of substantial, prolonged immunosuppression seems to be the main predisposing factor. Thus, a particularly high incidence of post-transplant lymphomas (PTL) was noted in patients receiving cyclosporin A, FK 506 and antibody OKT3 treatment.[4,9]

The majority of PTL are of B-cell origin, associated in most cases with Epstein-Barr virus (EBV) infection;[10,11] while, in contrast, T-cell PTL are uncommon and mainly EBV-negative.[12] Primary EBV infection leads to latent infection of B-cells, expressing part of the viral genome with episomal EBV persistence. The control of these cells is dependent on cytotoxic T-cells. Deliberate partial suppression of T-cell function by immunosuppressive drugs, in order to prevent graft rejection, is believed to result in an uncontrolled EBV-driven proliferation of B-cells. As proliferation continues, B-cell clones with structural alterations enabling autonomous growth escape immune control

inducing a lymphoproliferative disease.[13]

The overall outcome of patients with PTL is poor and about 50% of these patients die within a short period after diagnosis.[5,14] Prognostic factors are still not defined and there is no uniform treatment strategy.

Our aim was to describe the clinical presentation, pathologic features, treatment and outcome of all cases of PTL diagnosed in our hospital over the past 20 years. We also estimated the incidence of this complication and the risk of lymphoma compared to that observed in the general population.

Design and Methods

Patients

The analysis included all patients with PTL diagnosed from 1979 to 1999 at the Ciutat Sanitaria i Universitaria de Bellvitge, a 1,000-bed teaching institution in Barcelona, Spain that serves as a referral transplant and cancer center for an area with a population of more than 1 million adult inhabitants.

Between January 1979 and December 1999 a total of 1,860 solid organ transplants were performed in our hospital: 1,173 renal, 547 hepatic, and 140 cardiac transplants. Seventeen of the transplant recipients developed a PTL. Information regarding clinical, laboratory, and evolution data were extracted from the medical records and from files of the hospital's lymphoma registry.

All diagnostic biopsy material was reviewed by our pathologist. Morphologic and immunohistochemical studies were performed in formalin-fixed, paraffin-embedded tissue. PTL were classified according to the REAL classification; those cases seen prior to the date of publication of the REAL classification were reclassified. Cases diagnosed as polyclonal lymphoproliferative disease were excluded from the study, in order to avoid a selection bias.

In situ hybridization for the detection of Epstein-Barr encoded RNA (EBER) was performed on involved paraffin-embedded tissues using commercially available reagents (Dako, Denmark).

Definitions

Outcome was assessed by response to therapy, remission duration, and survival. Complete remission was defined as no evidence of disease by standard laboratory, radiographic, or histopathologic parameters; partial remission was defined as at least a 50% decrease of the perpendicular diameters of all measurable disease sites, without the appearance of new lesions. Disease-free survival was defined as the time from documentation of a complete remission to relapse or death. Overall survival was defined as the time from diagnosis of the lymphoproliferative disorder to death.

Statistical analysis

Results of the descriptive analysis are expressed as means and standard deviations (SD) for continuous data and number of cases with their proportion for qualitative data.

The average annual incidence rate of PTL was analyzed at 5-year intervals from 1979 to 1999, inclusive. PTL incidence rates were calculated using the number of new cases of PTL observed as the numerator and the person-years at risk during the study period as the denominator. Relative risks (RR) were estimated by comparing the incidence of PTL among transplanted patients with the incidence of lymphoma in the general population of the region (CIFC Vol 7).[15] To control for age differences between transplanted patients and the general population, incidence rates were age-standardized.

Survival analysis was performed at the univariate level by means of Kaplan-Meier techniques to estimate overall survival. All variables were individually evaluated in a hazard ratio model. Variables significantly related to survival were included in a multivariate Cox proportional hazards regression model. Results of the regression analyses were expressed as a hazard ratio (HR), with its 95% confidence interval (CI). Values of $p<0.05$ were considered to be statistically significant.

Results

Incidence and relative risks

Between 1979 and 1999, 17 of 1,860 transplanted patients (11 men and 6 women) developed a PTL. This is an incidence of 0.9% for the whole 20-year study period. The observed incidence of PTL among the recipients of different types of transplant are shown in Table 1. The annual incidence rate of PTL was 43.5 per 10^5 transplants, which is an almost 8-fold higher risk of lymphoma than in the general population. The risk of lymphoma was highest in those patients receiving a heart transplant, with an incidence rate of 357 per 10^5 heart transplants and a 35-fold higher risk than in the general population. This risk increased up to 12 times when multiple myeloma and Hodgkin's disease were excluded from the analysis. The incidence of PTL was stable throughout the study period (p=0.79).

Table 1. Incidence of post-transplant lymphoma by transplanted organ.

Transplanted organ	*Period*	*No. of transplants*	*PTLD (%)*	*AI $\times 10^5$*	*RR (95%CI)**
Kidney	1978-99	1173	8 (0.7)	32.5	5.8 (2.5-11.5)
Liver	1984-99	547	5 (0.9)	60.9	7.6 (2.5-17.8)
Heart	1991-99	140	4 (2.9)	357.1	35.7 (9.7-91.3)
Total	1978-99	1860	17 (0.9)	43.5	7.9 (4.6-12.7)

**Reference comparison with the regional rate of lymphomas in Tarragona, Spain. CIFC Vol. VII.[15] Abbreviations: AI: annual incidence; RR: relative risk.*

Clinical presentation and pathology

The main clinical and pathologic features of the patient population with PTL are summarized in Table 2. The mean age at time of diagnosis was 53±12 years (range: 27-73), and the mean time between transplantation and diagnosis of PTL was 31±29 months (range: 1-103). The median interval between transplantation and PTL was shorter in heart recipients (median 11 months; range=8-16 months) than in liver transplants (median 21 months; range 4.5-68 months) or renal transplants (median 50 months; range 1-103 months). This interval was also shorter after a second transplant (n=3). PTL developed during the first year in 7 patients (41%).

As far as concerns histologic subtype, the 17 cases of PTL comprised 4 polymorphic B-cell lymphomas, 3 large B-cell lymphomas, 2 mucosa associated lymphomas (MALT), 2 Burkitt's lymphomas, 2 plasmacytoma/multiple myeloma, 2 pleomorphic B-cell lymphomas, 1 Hodgkin's lymphoma, and 1 Sézary's syndrome. Immunophenotypic studies showed that 15 patients expressed markers of B-cell lineage (88%), 1 Hodgkin's disease and only 1 expressed T-cell lineage markers.

Staging and sites of involvement are also summarized in Table 2. Of the 17 patients, 8 (47%) had stage III or IV disease. Isolated extralymphoreticular disease was the most common presentation (n=16), and the most frequently involved site was the allograft (35%). No patient in our series presented central nervous system involvement.

EBV infection was evident in 7 of the 12 patients tested (58%). In all of these cases, the PTL was of B-cell origin.

Therapy and outcome

Therapeutic approach and response are summarized in Table 3. After transplantation and before the development of PTL, patients received immunosuppressive therapy for periods ranging from 1 to 103 months (median 16 months). All patients were treated with prednisone and cyclosporin A and some of them received additional immunosuppressive drugs: azathioprine (4 patients), antithymocyte

Table 2. Clinical features of patients with post-transplant lymphomas.

Patient	*Age (yrs)/Sex*	*Organ transplanted*	*Months between graft-PTLD*	*Histology*	*Tumor sites*	*Stage*	*LDH level*	*EBV*
1	61/M	Liver	4.5	Plasmocytoma	Liver	IV-B	14.7	NA
2	57/M	Kidney	44	MALT	Stomach	I-A	NA	–
3	48/M	Kidney (×2)	103/23	Myeloma	Maxillary bone	II-A*	68	NA
4	65/F	Liver	29	Diffuse large B-cell	Parotid gland	II-B	5.8	–
5	49/F	Liver	68	Burkitt's	Diffuse	IV-B	NA	+
6	45/M	Kidney	4	Polymorphic B-cell	Kidney	IV-B	NA	+
7	73/F	Kidney	14.5	Diffuse large B-cell	Kidney	IV-A	7.5	+
8	64/M	Kidney (×2)	59/39	Hodgkin	Liver	IV-B	6.9	–
9	33/M	Kidney	62	Polymorphic B-cell	Lung	I-A	NA	+
10	58/F	Heart	16	Sézary's syndrome	Skin	II-A	12.6	–
11	68/F	Liver	21	MALT	Orbit	I-A	5	NA
12	51/M	Liver (×2)	10.5/1	Diffuse large B-cell	Liver	IV-B	NA	–
13	60/M	Heart	8	Pleomorphic B- cell	Skin	I-B	11.3	+
14	38/M	Heart	8	Polymorphic B-cell	Lung	I-A	6.6	NA
15	59/M	Heart	15	Polymorphic B-cell	Intestinal	IV-A	10.3	+
16	53/F	Kidney	56.5	Burkitt's	Submaxillary gland	II-A	8.1	+
17	27/M	Kidney	1	Pleomorphic B-cell	Kidney, liver	IV-B	NA	NA

*M= Male; F= Female; ×2= Double transplant; Staging according to Ann Arbor classification and *Durie and Salmon. LDH normal range: 0.1-7.5 µkat/L. NA= Not available.*

Table 3. Treatment and outcome of the patients with post-transplant lymphomas.

Patient	*Immunosuppression (drug stopped)*	*Antiviral*	*Surgery*	*Chemotherapy*	*Radiotherapy*	*Outcome*
1	CSA + Azathioprine		-	-	CHOP (1)	Died progression 0.5 months
2	↓ CSA	-	Gastrectomy	-	-	CR +80 months
3	CSA	-	-	CNOP (6)	Local	Died progression 23 months
4	CSA	-	-	CHOP (6)	Local	Died progression 6 months
5	CSA + Azathioprine	-	-	Leukemia-like (1)	-	Died progression 2 months
6	CSA + Cellcept	-	-	-	-	Died progression 11 days
7	↓ Cellcept	-	-	CHOP (4)	-	PR + 25 months
8	↓ CSA	-	-	MOPP (2)	-	Died toxicity 2 months
9	CSA	-	Resection	-	-	Died progression 12 months
10	CSA	-	-	Chlorambucil + PUVA	-	CR +28 months Died myocardial infarction
11	No	-	-	-	Local	CR + 8 months
12	↓ CSA	-	-	-	-	Died progression 1 month
13	↓ CSA	Acyclovir	Resection	-	-	CR + 50 months
14	↓ Prednisone	-	Resection	CHOP (6)	-	CR + 77 months
15	Azathioprine +↓ CSA	-	Resection	CHOP (6)	-	CR + 75 months
16	↓ CSA	-	-	-	Local	CR + 52 months
17	CSA	-	-	-	-	Died progression and sepsis 2 months

↓ = tapered; CR= complete remission; PR= partial remission; (number of cycles). Leukemia-like= High intensity, brief duration chemotherapy. McMaster ML. J Clin Oncol 1991.

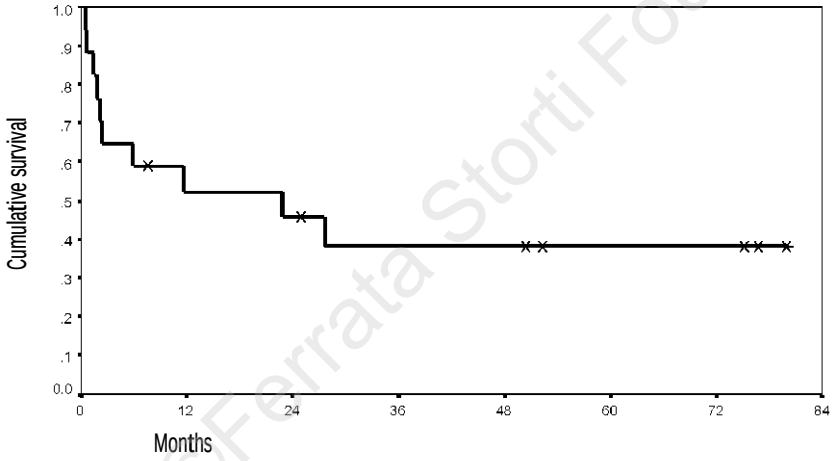

Figure 1. Overall survival among patients who developed PTL.

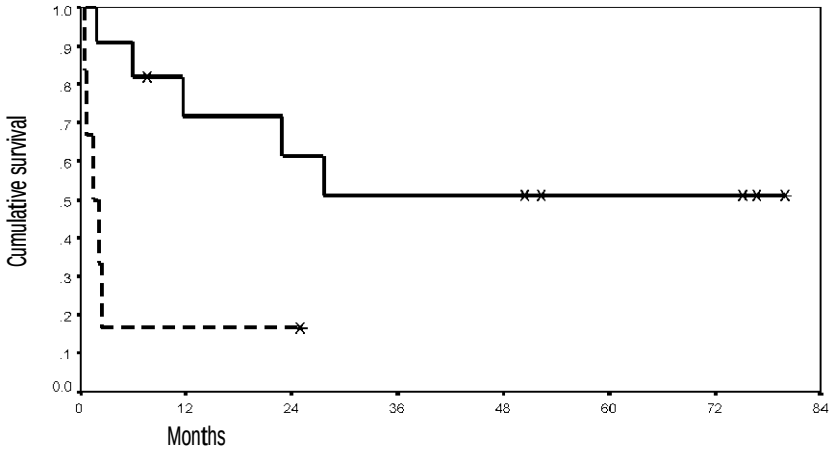

Figure 2. Overall survival among patients who developed PTL by site of lymphoma. (solid line: graft not involved; dashed line: graft involved by PTL).

globulin (ATG) (3 patients), azathioprine and ATG (6 patients), cellcept (2 patients), and OKT3 (1 patient).

As shown in Table 3, in 16 patients immunosuppressive therapy was either modified or stopped once the PTL had been diagnosed. In the remaining case (patient #11) immunosuppression was not modified and the patient received only orbital radiotherapy. Fourteen patients received further treatment including antiviral therapy with acyclovir, local radiotherapy, surgery and chemotherapy. The most frequent chemotherapy scheme used consisted of CHOP-based regimens (6 patients).

Overall survival is depicted in Figure 1. The median survival time was 24 months.

Five out of 8 patients who presented with localized disease are alive and in complete remission, without relapses through the study period and with a median follow-up of 62 months (range 15 to 90 months). The majority of patients (five out of six) who had allograft involvement died of progressive disease within 2 months of diagnosis. Only one patient (#7) is in partial remission, with a persisting kidney allograft lesion and high LDH, but without clinical manifestations.

Cox proportional hazards regression models were used to evaluate the relationship between survival and graft involvement (HR = 5.3; 95% CI 1.4 - 20.7) (Figure 2). The other variables tested (age, sex, Ann Arbor classification, EBV, LDH, transplanted organ and time between transplant and PTL) did not retain significant correlations.

Discussion

Solid organ transplantation has become a widespread surgical intervention. The development of subsequent PTL remains one of the most severe complications in these patients. Although we have a better understanding of the biology and pathogenesis of these disorders, treatment is often ineffective and patients commonly die of this complication.

In this retrospective study on a cohort of 1,860 solid organ transplant recipients, 17 patients developed PTL (incidence 0.9%), which confirms the reports of this complication available in the literature.[1-8,10] The incidence of lymphoma differed significantly in relation to the organ allografted: kidney recipients had the lowest incidence rate (0.7%) and heart recipients the highest (2.9%). The overall relative risk of PTL was almost 8-fold greater than the risk of lymphoma in the general population, and was especially high in heart transplant recipients; this is most likely due to the more aggressive immunosuppression therapy used in these patients. We could not find differences in the frequency of PTL along the study period, although the immunosuppressive regimes varied.

The mean time between transplantation and the diagnosis of PTL in our study group was 31 months. However, the majority of PTL with graft involvement were diagnosed within the first year after transplantation. A higher incidence of lymphoproliferative diseases is reported in the literature to occur in the early months following transplantation. The majority of cases were EBV-related polyclonal proliferations, which were not included in our study.[8,14] We also found that heart recipients developed PTL earlier than kidney or liver transplant recipients. This is thought to be a consequence of the more intense immunosuppression received in this period and of the type of organ transplant.[1-5]

The histopathologic observations in our series comply with the general concept that the great majority of PTLs are of B-cell origin. In our study, PTL was related to EBV infection in about 50% of the cases tested, which is lower than that reported by other authors.[3,6,7,10,11] Moreover, we failed to detect EBV in 30% of the B-cell PTLs. Underdetection of EBV cannot be ruled out in this retrospective series, although *in situ* hybridization for EBV detection in our laboratory strongly correlated with detection of this virus in other laboratories. Several studies consider EBV-negative PTL as a distinct entity with late onset (>1 year after the transplant) and a histologic and clinical presentation similar to that of lymphomas that develop in immunocompetent subjects. This group of lymphomas might be related to other infectious agents or genetic changes.[16,17]

The outcome of our patients with PTL was poor. In our study, PTL at the site of the graft was the only statistically significant predictor of poor outcome. As other authors have reported, up to 20% of PTL can appear at the graft site.[1,18,19] In the majority of cases, PTL is an early complication after transplantation that, at times, results in a mistaken diagnosis of rejection because of difficulty biopsies and similar symptoms. A number of hypotheses have been formulated to explain this predisposition to develop PTL in the graft: the large amount of lymphoid tissue in these locations, stimulatory factors released during the transplant procedure

and differences in EBV serologies of donor and recipient. The outcome of these patients is poor because the PTL is difficult to treat, it progresses rapidly and the graft is frequently lost. Other clinical and laboratory data related to survival elsewhere described[14] did not show an association in our study, probably because of the small number of cases.

It is likely that early diagnosis and treatment may improve the outcome of these patients. There are groups working on monitoring EBV DNA load in blood in order to identify those post-transplanted patients at risk of developing a lymphoproliferative disease.[20] Early identification of patients at high risk, together with a temporal reduction of immunosuppression and a specific therapeutic approach could reduce PTL-related morbidity and mortality in these patients. Reduction of immunosuppression has been of great value in early-diagnosed post-transplant lymphoproliferative diseases, especially polyclonal ones. The use of specific treatments such as anti B-cell antibodies[21,22] and EBV-specific cytotoxic T-lymphocytes,[23] are therapeutic approaches with fewer side effects than those observed using classic chemotherapy. It is likely that the introduction of these new regimes will improve survival. Further progress in the prevention and treatment of this group of diseases will require prospective clinical trials and standardized treatment.

Contributions and Acknowledgments

EDD and SS were responsible for the conception of the study, clinical data, statistical analysis, and writing of the article. EGB was responsible for the writing of the article. VR and ADC perfomed the pathologic and cytological studies, and Epstein-Barr virus detection. SGV, JF, and NM were responsible for the care of the patients. BO collected the clinical data. JP, AG, and AFS were responsible for the review of the paper. All authors gave their approval to the final version of the paper.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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Potential implications for clinical practice

Patients receiving a solid organ transplant have almost an 8-fold higher risk of lymphoma than the general population. The mortality caused by lymphoma localized to the site of the graft is up to five times higher than the mortality due to disease in other locations.

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