Research paper

haematologica 2002; 87:67-77
http://www.haematologica.it/2002_01/067.htm

Post-transplant lymphoproliferative disorders: improved outcome after clinico-pathologically tailored treatment

GIULIANA MUTI,* SILVIA CANTONI,* PIERLUIGI ORESTE,° CATHERINE KLERSY,# GUIDO GINI,* VALENTINA ROSSI,* GIOVANNA D'AVANZO,* PATRIZIA COMOLI,@ FAUSTO BALDANTI,^ MARCO MONTILLO,* ANNAMARIA NOSARI,* ENRICA MORRA,* FOR THE COOPERATIVE STUDY GROUP ON PTLDS[1]

*Division of Hematology, °Institute of Pathology, Niguarda Hospital, Milan; #Biometry and Clinical Epidemiology, @Pediatric Science Department, ^Institute of Virology, IRCCS Policlinico San Matteo, Pavia, Italy

Correspondence: Giuliana Muti, MD, Department of Oncology-Haematology, Niguarda Hospital, Piazza Ospedale Maggiore, 3 20162 Milan, Italy. Phone: international +39.02.64442210. Fax: +39.02.64442033. E-mail address: ematologia@ospedale-niguarda.it

Background and Objectives. Clinical and pathologic variability of post-transplant lymphoproliferative disorders (PTLDs), their aggressive behavior and the recognized therapy-related toxicity make management of patients with these disorders difficult. Assessment of first-line treatment and identification of prognostic factors need to be better defined.

Design and Methods. Data on 40 PTLDs which developed in adult solid organ recipients were analyzed in order to evaluate clinical and pathologic features, response to treatment and prognostic factors. Data were collected retrospectively between 1989 and 1996; since 1997 a prospective study has been activated.

Results. The median time from transplant to PTLD was 56 months. Regarding histologic features, plasmacytic hyperplasia was diagnosed in 5 patients (12.5%), polymorphic lymphoproliferative disorders in 3 (7.5%), malignant lymphoma in 32 (80%). The diagnosis was made at autopsy in eight patients (20%). Late-onset PTLDs (>12 months from transplant) occurred in 33 patients (83%), EBV-negative forms in 12 (31%). Relevant differences have been observed between EBV-positive and EBV-negative forms. Twenty-nine patients completed their scheduled treatment and are evaluable for outcome. The cumulative probability of survival at 1 year is 57% (CI 37.6-73.4) and the median survival time of the entire group has not been reached at 54 months. Clinical stage, performance status, lactate dehydrogenase and number of sites are predictive factors for survival. The International Prognostic Index and the PTLD index are able to identify different risk groups.

Interpretation and Conclusions. Although rare, PTLDs are a significant cause of mortality in allograft recipients. Therapy tailored on histologic and clinical features of PTLD is feasible and is able to give long-lasting complete responses.

Key words: PTLD, post-transplant lymphoproliferative disorders, Epstein-Barr virus.

Post-transplant lymphoproliferative disorders (PTLDs) are a severe complication arising in solid organ transplant patients; their reported incidence ranges from 1% to 20%,[1-3] according to factors such as type of organ transplanted, variance in immunosuppressive protocols - with special reference to the use of OKT3 antiserum – and pre-transplant immune status for Epstein-Barr virus infection; [2-10] recently, cytomegalovirus (CMV) disease was identified as an additional risk for development of PTLD.[11]

In most cases Epstein-Barr virus (EBV) proteins (i.e. LMP, EBNA) or EBV-encoded RNA (EBER) are detectable in pathologic specimens: these cases, termed EBV-positive PTLDs, are considered the result of a multistep oncogenetic process triggered by EBV in chronically immunosuppressed patients. However, chronic immunosuppression in itself represents a relevant risk factor for the development of a variety of neoplastic diseases, especially those rare

[1]Cooperative Study Group on PTLDs: Maria Frigerio,* Fabrizio Oliva,* Ghil Busnach,° Enrico Minetti, ° Andrea De Gasperis,# Alberto Alberti,# Gianfranco Rondinara,# Emanuela De Juli,@ Giorgio Zilio,@ Livio Gargantini,^ Roberto Cairoli,^ Laura Pezzetti,^ Liliana Intropido,^ Edgardo Bonacina,§ Ernesto Minola,§ Silvio Veronesi,§ Marcello Gambacorta§ (*Heart, °Kidney, #Liver, @Lung Transplant Units, ^Division of Hematology, §Institute of Pathology, Niguarda Hospital, Milan, Italy).

in the general population.[12] This epidemiological risk emphasizes the uncertain assessment of EBV-negative PTLDs, that is, whether they represent a true PTLD lacking EBV markers, or a distinct entity with oncogenetic pathways other than EBV (e.g. HHV8, HCV), or rather a conventional lymphoma occasionally arising in transplanted patients.[13-16]

The clinical variability of the disease (rapid or sudden onset and aggressive progression), frequent extranodal involvement, and wide spectrum of histologic features (from reactive polyclonal hyperplasia to monoclonal monomorphic forms) make the diagnosis of PTLDs difficult; moreover PTLDs may have a multicenter origin and different clonally distinct tumors may coexist in different sites in the same patient.[17]

The aggressive behavior of PTLDs and the recognized therapy-related high toxicity make management of transplanted patients with PTLD complex and far from optimal. Moreover, so far, the low number of patients, and the differences in therapeutic approach reported in the literature have not allowed assessment of first-line therapy and have made the identification of prognostic criteria difficult.[1,3,14,18-23]

In this study data on 40 PTLDs which developed in adult solid organ recipients were analyzed in order to evaluate clinical and pathologic features, response to treatment and prognostic factors.

Design and Methods

Study design

All patients with a diagnosis of PTLD made either at autopsy or *in vita* at our hospital between February 1989 and June 2001 were included in this study. For patients diagnosed before 1997 data were collected retrospectively; since January 1997 a prospective clinical study has been underway.

Patients and immunosuppressive protocols

Our study comprises 22 heart, 11 kidney, 5 liver and 2 lung recipients who developed PTLD; all but three patients were transplanted at our hospital between 1973 and 2000.

Immunosuppressive regimens used comprised a combination of anti-lymphocyte globulin, cyclosporin A (since1983), azathioprine and prednisone. None of the patients received OKT3 as induction therapy. Tacrolimus (since 1995) and mycophenolate mofetil (since 1998) replaced cyclosporin A and azathioprine, respectively, in selected patients. After biopsy diagnosis, patients underwent standard staging, comprising complete blood chemistry, total body computed tomography (CT) scans, bone marrow biopsy and aspirate. Special diagnostic procedures (gastrointestinal studies, central nervous system CT or magnetic resonance, etc.) were performed when clinically indicated.

Patients were staged according to the Ann Arbor classification. Performance status was assessed using the Eastern Cooperative Oncology Group (ECOG) scale.

PTLD was classified as early-onset (≤ 12 months from transplantation) or late-onset (> 12 months) according to the definition of Armitage.[9]

Pathology and virology

A single pathologist (P.O.) reviewed all diagnostic biopsy and autopsy specimens. For morphologic examination, tissue specimens were fixed in 10% buffered formalin and embedded in paraffin; in selected cases, some specimens were plastic-embedded to optimize morphologic examination. Specimens from autopsies were collected 24-48 hours *post-mortem* and processed similarly. Tissue sections were stained with hematoxylin-eosin and Giemsa. The diagnosis of PTLD and its division into three categories – namely plasmacytic hyperplasia (PH), polymorphic lymphoproliferative disorder (PLD) and malignant lymphoma/plasmacytoma-like (ML) – were based upon criteria established by Knowles *et al.*[24] Whenever possible, morphologic classification of ML was made according to the criteria of the Revised European American Lymphoma (REAL) classification.[25] However, when considered more appropriate, the International Working Formulation classification was also used to subclassify ML. Accordingly, most of the diffuse large B-cell lymphomas of the REAL classification were subclassified as immunoblastic lymphomas.

The immunophenotypic profiles were assessed on paraffin-embedded tissue sections using the streptavidin-alkaline phosphatase (SAP) technique.

In situ hybridization studies for EBV-encoded RNA (EBER) were performed on paraffin-embedded tissue sections using EBER1 and EBER2 (EBER PNA) oligonucleotide PNA probes. The presence of EBV was subsequently verified by polymerase chain reaction (PCR). The presence of HTLVI and rearrangement of immunoglobulin genes, as evidence of B-cell monoclonality, were likewise verified by PCR.

Since 1998 both EBV DNA copies in peripheral blood lymphocytes and interleukin 10 (IL10) were included among the laboratory tests performed in transplanted patients with ill-defined symptoms raising a suspicion of PTLD. EBV DNA copies were quantified by PCR,[26] and IL10 was determined by an ELISA assay, sensitive to human as well as to viral IL10.

Treatment of PTLD

The first step in the management of PTLD in all patients diagnosed *in vita* was reduction of the immunosuppressive regimen: azathioprine was discontinued and cyclosporin A was reduced in order to obtain 50% of therapeutic plasma levels; none of the patients with PTLD was receiving either tacrolimus or mycophenolate mofetil at the time of diagnosis.

Chemotherapy (single agent - i.e. cyclophosphamide - or polychemotherapy - i.e. CVP: cyclophosphamide, vincristine, prednisone; CHOP: cyclophosphamide, adriamycin, vincristine, prednisone; VACOP-B: etoposide, adriamycin, cyclophosphamide, vincristine, bleomycin, prednisone; DHAP: dexamethasone, cytarabine, cisplatin) was tailored according to histologic subtype and disease burden. Because of the increased cardiotoxicity of anthracyclines seen in some patients,[27] heart transplant recipients received reduced-dose adriamycin (≤120 mg/m^2 cumulative dose); in lung transplant patients methotrexate substituted bleomycin in order to avoid lung toxicity.

Since 1997 therapeutic regimens have also comprised high-dose immunoglobulins (HDIg) and antivirals (acyclovir) for all EBV-positive PTLDs.

In the last two years monoclonal antibody (rituximab) has been introduced in the therapeutic schedule for CD20-positive patients with limited (stage I-II) or polyclonal disease.

Since 2000 autologous cytotoxic T-lymphocyte (CTL) lines have been prepared for therapeutic use in all EBV-positive PTLD patients: EBV-specific CTLs were reactivated from the patient's peripheral blood mononuclear cells and expanded *in vitro* according to a method previously reported,[28] following GMP standard procedures.

Whenever clinically indicated, surgery and/or radiation therapy were employed too, either alone or in combination with other treatments.

Radiofrequency tissue ablation was employed in a single patient (a liver recipient), in place of conventional radiotherapy, contra-indicated because of the particular site of disease (hepatic hilum).

Patients diagnosed as having low-grade gastric MALT lymphoma were managed with standard anti-*Helicobacter* therapy (omeprazole, clarithromycin, metronidazole), similarly to their immunocompetent counterparts.

During chemotherapy administration, reduced-dose immunosuppression was maintained (no azathioprine; cyclosporin A plasma levels at 50% of therapeutic range). At completion of chemotherapy, immunosuppression was reintroduced maintaining cyclosporin A plasma levels at 50-75% of the therapeutic range; patients also received low-dose prednisone.

Statistical analysis

Descriptive statistics were computed as mean and standard deviation (SD) for continuous variables, or median and quartiles in the cases of skewed distribution, and as absolute and relative frequencies for categorical variables. The cumulative probability of survival was computed by means of Kaplan Meier estimation. Survival was compared for a series of potential risk factors by means of a log-rank test. Hazard ratios (HR) and their 95% confidence intervals were computed by using a Cox model, after checking that the proportional hazard assumption was verified. No multivariate model was fitted due to the low number of events. Finally a series of characteristics were compared according to year of diagnosis (before or after 1997) by means of the Mann Whitney U-test for continuous variables and Fisher's exact test for categorical variables. A two-sided p-value <0.05 was considered to be statistically significant. Stata 7 software (Stata Corp, College Station, TX, USA) was used for the computations.

Prognostic risk was also evaluated according to the criteria of both the International Prognostic Index (IPI) for non-Hodgkin's lymphomas in immunocompetent patients,[29] and the PTLD Index recently proposed by Leblond *et al.*[14] The two different prognostic indices have been compared with respect to their predictive ability by calculating Maddala-explained variation, based on Cox models.

Results

Time to diagnosis and epidemiological characteristics

The clinical and pathologic characteristics of the PTLD patients are reported in Table 1. The median time from transplant to PTLD was 56 months (interquartile range-IQR 27-87); early-onset PTLD was diagnosed in 7 patients (17.5%): 4 liver, 2 heart, and 1 lung recipients. Over a 12-year period (from 1989 to 2001) some epidemiological and clinical characteristics changed (Table 2): in the retrospective part of study (1989-1996), 11 PTLDs were diagnosed (1.5 new diagnoses/year), 4 at autopsy (36%) and 7 *in vita*. From January 1997 until June 2001 (prospective study) 29 new cases were diagnosed (6.4 diagnoses/year), 4 at autopsy (14%) and 25 cases *in vita*. Moreover, in the earlier period, the median time from transplant to PTLD was 27 months (IQR

Table 1. Pathologic and clinical features of the 40 PTLD patients.

Pt no	Age(y)/sex	Graft	Time from Tx to PTLD (m)	Clonality/morphology	ECOG (PS)	Stage§	EBER	EBV DNA copies°	IL10 (pg/mL)	PTLD sites
1	56/M	Heart	5.5	mono B/ML (immunobl)	*	IV	+			Ln.Lu.K.Li.BM
2	38/M	Liver	1.5	null/ML (ALCL)	*	II E	+			Lu
3	47/F	Heart	30	mono B/ML (immunobl)	*	IV	+			Ln. GI.Pa
4	34/F	Kidney	59	mono B/ML(immunobl)	1	I E	+			GI
5	54/M	Kidney	39	mono B/Plasmoc. like	3	IV	nd			BM
6	68/M	Heart	48.5	mono B/ML(immunobl)	*	IV	+			Ln.BM.S
7	55/M	Heart	79	mono B/ML(immunobl)	4	IV	+			Ln.BM.Li.S
8	46/M	Lung	4.5	mono B/PLD	2	IIE	+			Lu
9	54/M	Kidney	77	null/ML(ALCL)	2	III	-			Ln
10	58/M	Heart	17	mono T/ML(pleomorphic)	3	IV	-			Ln.Sk.H.Li.Lu.GI
11	45/M	Liver	10	mono B/ML(high grade)	1	I E	-			Li
12	55/F	Kidney	64	mono B/ML(immunobl)	2	II E	-			Ln.GI.
13	23/M	Heart	27	poly B/PH	0	I E	+	100,000		Sk
13a^	25/M	Heart	49	poly B/PLD	2	III	+	10,000	47	Ln.S
14	67/M	Kidney	36	mono B/ML(immunobl)	*	IV	+			Ln.K.Li.H.T.GI
15	67/M	Heart	6.5	mono B/PLD	1	IIE	+			Lu
16	54/M	Heart	38	mono B/ML(Burkitt)	4	IV	+			K.Li.Lu.BM.H
17	63/F	Kidney	174	mono T/ML(pleomorphic)	*	IV	-			Ln.Li.S
18	45/F	Liver	63	mono B/ML(immunobl)	4	IV	+	1,000	140	Ln.GI.Per
19	57/M	Heart	92	mono B/ML(Burkitt)	4	IV	+	5,000	28	Ln.GI.K.Lu.CNS
20	60/M	Kidney	53	mono B/ML(MALT)	1	I E	-	<10		GI
21	53/M	Kidney	72	mono B/ML(immunobl)	*	III	+			Ln
22	62/M	Heart	108	mono B/ML(immunobl)	1	I E	+	5,000	5	Sk
23	41/M	Heart	81	mono B/ML(DLCL)	1	III	-	<10	57	Ln.S
24	26/M	Heart	113	mono B/ML(MALT)	1	I E	-	500	1	GI
25	70/M	Heart	61	mono B/Plasmoc. like	1	I	-	30	2	Ln
26	47/M	Heart	28	mono B/ML(immunobl)	3	IV	+	10	1	Ln.GI
27	67/F	Kidney	103	mono B/ML(DLCL)	2	II	-	<10		Ln.S
28	42/M	Heart	72	mono B/ML(high grade)	3	IV	+	1,000	40	Ln.BM.Li.K.S
29	60/M	Heart	43	mono B/ML(high grade)	2	IV	+	100	620	Ln.S.Sk.GI
30	34 /M	Liver	7	mono B/ML(high grade)	1	IV	+	200	19	Ln.Li
31	68/M	Heart	54	mono B/Plasmoc. like	3	IV	-	<10	1	Ln.BM
32	61/F	Kidney	14	mono B/ML(ALCL)	1	IV	+	100	21	Sk
33	32/M	Heart	57	mono T/ML(LGL)	1	IV	-	<10	14	S. BM
34	54/M	Heart	35	poly B/PH	*	IV	+			Ln.S.Li.K.H.BM.Lu
35	60/M	Heart	145	poly B/PH	1	IV	+	200	2	Ln.Li.S
36	62/M	Heart	128	mono B/ML(Burkitt-like)	4	IV	+			Ln.S.K.GI.BM
37	59/M	Liver	5	mono B/PLD	1	IE	+	10	5	Li
38	36/M	Heart	106	poly B/PH	1	III	+	100	5	Ln
39	39/M	Kidney	133	monoB/ML(high grade)	4	IV	+	30,000		Ln.BM.Li.K.GI
40	38/M	Lung	126	poly B/PH	1	IV	+	3,000	490	Ln.S.BM
40a^	38/M	Lung	126	mono B/ML(high grade)	2	IV	+			Ln.S.BM

*M. male; F. female; Tx. transplant; ECOG (PS). Eastern Cooperative Oncology Group (Performance Status); nd. not done; ML. malignant lymphoma; PH.plasmacytic hyperplasia; PLD. polymorphic lymphoproliferative disorders; Ln. lymph nodes; Lu. lung; K. kidney; Li. liver; BM. bone marrow; GI. gastrointestinal; Pa. pancreas; S. spleen; Sk. skin; H. heart; T. thyroid; Per. peritoneum; CNS. central nervous system. §According to Ann Arbor; ^letters indicate subsequent biopsies; *autopsy diagnosis; °EBV DNA copies/10^5 PBL.*

5-60), and 36% were early-onset forms, while, in the latter, the median time from transplant to PTLD was 64 months (IQR 37-107), and only 10% were early-onset forms.

Pathologic and virologic studies

Data on the histologic and biological features of PTLD are shown in Table 1. Five cases (#13, 34, 35, 38, 40) were classified as having polyclonal PH, and 3 cases (#8, 15, 37) as monoclonal PLD; in the remaining 32 patients monoclonal ML was diagnosed. Two years after diagnosis, one patient (#13) shifted from having polyclonal PH to polyclonal PLD. In one patient (#40), two nodal biopsies performed at two weeks' interval demonstrated two different histologic features: the first one was consistent with polyclonal PH, the second one with monoclonal ML; in this case multicenter disease, rather than histologic progression, was suspected.

Both MALT lymphomas (#20, 24) were low-grade EBER-negative lymphomas and both stained positive for *Helicobacter pylori*. T-cell lymphoma was diagnosed in three cases (7%), and all were EBER and HTLV-I negative.

Twelve of 39 tested patients (31%) were EBER-negative; in all these patients, pathology was consistent with monoclonal ML and all but one were late-onset forms.

No differences emerged between histologic and biological characteristics of PTLD patients diagnosed before or after 1997: in particular, the prevalence of EBV-negative forms was the same (30% and 31%, respectively).

EBV DNA load and IL10

Twenty-two patients (15 EBER-positive and 7 EBER-negative) were tested for EBV DNA copies in peripheral blood lymphocytes: overall, the median value observed was 150 copies/10^5 PBL (IQR 10-1000); however, in EBER-positive patients the median value was higher than in EBER-negative forms (1000/10^5 and <10/10^5 PBL, respectively).

IL10 was tested at diagnosis in 18 patients (13 EBER-positive and 5 EBER-negative): the median value was 16 pg/mL (IQR 1-57); again, EBER-positive patients had the higher levels (median value 21 vs 2 pg/mL) (Table 1).

Clinical features

In our series (Table 2), extranodal involvement was frequent with an overall prevalence of 83%: gastrointestinal tract (32%), liver (32%), and bone marrow (25%) were the most commonly involved sites, usually in the context of widespread disease. The CNS was involved in one patient only, diagnosed with Burkitt's lymphoma (#19). Graft involvement, in both isolated and widespread disease, was more frequent in early-onset (57%) than in late-onset forms (15%).

Table 2. Epidemiological and clinical characteristics according to the period of diagnosis.

	Year of diagnosis		
	1989-1996	*1997-June 2001*	*p*
No. diagnosis	11	29	
Diagnosis/year	1.5	6.4	
Median time from Tx to PTLD (mos)	27	64	0.01
No. Early-onset (%)	4/11 (36)	3/29 (10)	0.07
No. EBER neg (%)	3/10 (30)	9/29 (31)	1.00
No. autopsy diagnosis (%)	4/11 (36)	4/29 (14)	0.18
No. early deaths (%) (median time 10 days)	2/11 (18)	3/29 (10)	0.6

Eight patients (20%) were diagnosed at autopsy and all but one of them - a liver recipient - had widespread disease. Patients diagnosed *in vita* (32/40) had prevalently widespread disease (63% stage III-IV vs 37% stage I-II) while they were similarly distributed with respect to performance status (50% ECOG 0-1 and 50% ECOG 2-4).

EBV-negative versus EBV-positive patients

Compared to EBV-positive forms, in our series EBV-negative PTLDs occurred later (median time 61 vs 43 months) and all were malignant lymphomas, while EBV-positive PTLDs included other histologic forms. Moreover, compared to EBV-positive forms, at diagnosis EBV-negative cases more frequently presented with limited (stage I-II) disease (50% vs 26%, respectively); extranodal involvement was less frequent (58% vs 92%); performance status was better (ECOG 0-1: 50% vs 37%); moreover, autopsy diagnoses were less frequent (8% vs 26%).

Treatment and outcome

Three patients (# 4,5,35) were transferred after diagnosis to their local referral hospitals, and are not evaluable; one patient (# 40) is currently on therapy. Twenty-nine patients are evaluable for outcome (Table 3).

Five patients died early (median time 10 days from diagnosis - range 1-18), before any additional treatment, besides reduction of immunosuppressive regimen, could be instituted.

A chemotherapy-based regimen was the first-

Table 3. Treatment and outcome of the 40 PTLD patients.

		Treatments								
Pt no	*Morphol*	*<IS*	*Surgery*	*RT*	*Antiviral*	*HDIg*	*CT*	*Anti CD20*	*other*	*Outcome*
1	ML	-	-	-	-	-	-	-	-	Autopsy diagnosis
2	ML	-	-	-	-	-	-	-	-	Autopsy diagnosis
3	ML	-	-	-	-	-	-	-	-	Autopsy diagnosis
4	ML	-	-	-	-	-	-	-	-	Lost after diagnosis
5	ML	-	-	-	-	-	-	-	-	Lost after diagnosis
6	ML	-	-	-	-	-	-	-	-	Autopsy diagnosis
7	ML	yes	no	no	no	no	no	no	no	Dead at 10 days (related causes)
8	PLD	yes	no	no	yes	no	yes	no	no	Dead at 40 days (related causes)
9	ML	yes	no	no	no	no	yes	no	no	Dead at 120 days (related causes)
10	ML	yes	no	no	no	no	no	no	no	Dead at 18 days (related causes)
11	ML	yes	no	no	no	no	yes	no	no	Dead at 47 (related causes)
12	ML	yes	yes	no	no	no	yes	no	no	Dead at 480 days (unrelated causes) NED
13	PH	yes	yes	no	yes	no	no	no	no	Recurrent disease
13[a]	PLD	yes	no	no	yes	yes	yes	no	no	Alive at 1,643 days, NED
14	ML	-	-	-	-	-	-	-	-	Autopsy diagnosis
15	PLD	yes	no	no	yes	no	no	no	no	Alive at 1,618 days, NED
16	ML	yes	no	no	no	no	no	no	no	Dead at 10 days (related causes)
17	ML	-	-	-	-	-	-	-	-	Autopsy diagnosis
18	ML	yes	no	no	yes	yes	yes	no	no	Alive at 1127 days, NED
19	ML	yes	no	no	yes	yes	yes	no	no	Alive at 1,045 days, NED
20	ML	yes	no	no	no	no	no	no	yes[1]	Alive at 950 days, NED
21	ML	-	-	-	-	-	-	-	-	Autopsy diagnosis
22	ML	yes	no	yes	yes	yes	yes	no	yes[2]	Alive at 861 days, on therapy
23	ML	yes	yes	no	yes	yes	yes	no	no	Alive at 831 days, NED
24	ML	yes	no	no	no	no	no	no	yes[1]	Alive at 818 days, NED
25	ML	yes	no	no	no	no	yes	no	no	Alive at 795 days, NED
26	ML	yes	no	no	yes	yes	yes	no	no	Dead at 85 days (related causes)
27	ML	yes	no	no	no	yes	yes	no	no	Alive at 670 days, NED
28	ML	yes	no	no	yes	yes	yes	no	no	Alive at 642 days, NED
29	ML	yes	no	no	yes	yes	yes	no	no	Dead at 113 days (related causes)
30	ML	yes	no	no	yes	yes	yes	no	yes[3]	Alive at 589 days, NED
31	ML	yes	no	no	no	no	yes	no	no	Alive at 407 days, NED
32	ML	yes	yes	no	no	yes	yes	yes	no	Dead at 161 days (related causes)
33	ML	yes	yes	no	no	no	yes	no	no	Alive at 340 days, NED
34	PH	-	-	-	-	-	-	-	-	Autopsy diagnosis
35	PH	-	-	-	-	-	-	-	-	Lost after diagnosis
36	ML	yes	no	no	no	no	no	no	no	Dead at 1 day (related causes)
37	PLD	yes	yes	no	yes	yes	no	yes	no	Alive at 137 days,NED
38	PH	yes	no	no	yes	yes	no	no	no	Dead at 45 days (related causes)
39	ML	yes	no	no	no	no	no	no	no	Dead at 1 day (related causes)
40	PH	yes	no	no	yes	yes	no	no	no	Too early to evaluate
40[a^]	ML	yes	no	no	yes	yes	yes	no	no	Too early to evaluate

Abbreviations: ML: malignant lymphoma; PLD: polymorphic lymphoproliferative disorders; PH: plasmacytic hyperplasia; NED: no evident disease; Is: immunosuppression; RT: radiotherapy; HDIg: high dose immunoglobulin; CT: chemotherapy; [1]conventional anti-Helicobacter treatment (omeprazole + antibiotics); [2]autologous CTLs; [3]radiofrequency.

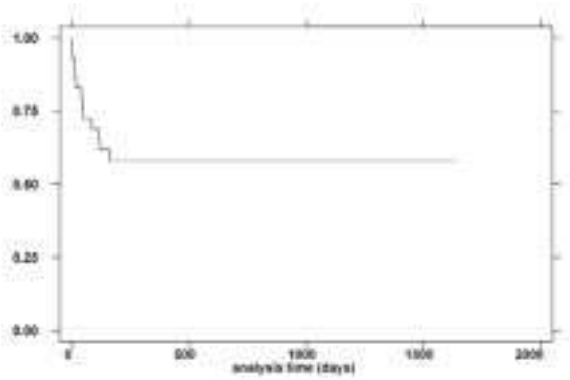

Figure 1. Kaplan-Meier survival estimate: 29 patients evaluable for follow-up.

line therapy in 17 cases, and second-line therapy in one patients; complete response (CR) was achieved in 12 (67%). One patient (# 22) relapsed after three months and developed recurrent disease, in spite of multiple lines of treatment. Good (stable disease) and long-lasting (25 months) response was subsequently achieved after combined treatment with chemotherapy, surgery, and infusion – every two weeks in the last 10 months - of autologous CTLs.

Antiviral agents and HDIg were first-line therapy in three patients (#13,15,38), 2 with polyclonal PH and 1 with monoclonal PLD; one patient achieved CR, one developed recurrent disease and entered CR after chemotherapy, and one patient died of an opportunistic infection.

Anti-CD20 monoclonal antibody was employed in two cases (#32,37); of these, the first patient, treated with rituximab plus chemotherapy, died of an infection; the second one underwent surgery followed by rituximab and achieved CR.

Conventional anti-Helicobacter treatment was able to induce CR in the two patients (#20, 24) with MALT lymphoma.

In our study, 16 of 23 (70%) patients completed scheduled treatment and entered CR; seven patients died during or at the end of treatment, at a median time of 85 days (range 40-161), because of disease progression or treatment-related toxicity. Notably, in our series no deaths occurred because of relapse or therapy-related late toxicity. The cumulative probability of survival at 1 year is 57% (CI 37.6-73.4) and the median survival time of the entire group has not been reached at 54 months (Figure 1).

Table 4. Potential risk factors analyzed for survival.

	Univariate analysis	
Variable	*HR (95% CI)*	*p value*
Ann Arbor, 3-4 vs1-2	3.96 (0.87-18.13)	0.04
PS, 2-4 vs 0-1	3.85 (1.04-14.28)	0.03
Sites > 1	4.47 (0.58-34.69)	0.08
Histology		0.96
Histology, PLD vs PH	0.91 (0.06-14.6)	0.94
Histology, ML vs PH	1.16 (0.15-9.14)	0.89
EBER pos	2.34 (0.63-8.67)	0.18
LDH > 1 n.v.	3.28 (0.83-12.95)	0.07
IL10 serum levels >6	0.77 (0.11-5.51)	0.8
EBV DNA copies >200	0.28 (0.03-2.50)	0,2
Chemotherapy	0.32 (0.10-1.00)	0.06
Surgery	0.25 (0.03-1.97)	0.11
Antiviral	0.53 (0.17-1.68)	0.28
Radiotherapy		0.27*
Alfa Interferon		0.27*
Monoclonal antibodies	0.73 (0.09-5.7)	0.76
HD Ig	0.44 (0.13-1.48)	0.17
other treatments	0.40 (0.05-3.12)	0.32
IPI index		0.03
IPI goup L-H vs L	7.32 (0.66-81.19)	0.1
IPI group I-H vs L	10.18 (1.18-87.82)	0.03
IPI group H vs L	11.31 (1.25-101.80)	0.03
PTLD index		0.08
PTLD group I vs L	3.32 (0.36-36.5)	0.3
PTLD group H vs L	6.51 (0.82-51.59)	0.07

*PS: performance status;*log-rank test.*

During chemotherapy administration (VACOP-B), 3 acute rejection episodes were observed, all responsive to high-dose corticosteroid administration (#19, 22, 28). Severe chronic rejection developed in three additional patients (#12, 18, 23) after completion of chemotherapy, while on reduced-dose immunosuppression. For one patient (#12), a kidney recipient, surgical removal of the allograft was necessary; this patient died of intestinal ischemic necrosis and sepsis two months after surgery with no signs of recurrence of PTLD at autopsy. The other two patients, a liver recipient and a heart recipient, received tacrolimus and are alive and well at 37 and 27 months, respectively, after diagnosis of PTLD.

Prognostic factors

The potential clinical and pathologic risk factors analyzed are listed in Table 4.

In univariate analysis only PS, stage III-IV, LDH >1 normal value and sites >1 increased relative risk of death by 3-4 times.

According to IPI criteria, 11 of 29 evaluable patients were assigned to the low-risk group, 4 to the low-intermediate group, 7 to the high-intermediate group and 7 to the high-risk group. According to PTLD index, 8 patients were assigned to the low-risk group (PS<2 and <two sites), 5 patients to the intermediate-risk group (PS ≥ 2 or two or more sites), and 16 patients to the high-risk group (PS ≥ 2 and two or more sites). Survival curves according to risk groups are shown in Figures 2 (IPI) and 3 (PTLD index). According to the IPI index, patients stratified into high and high-intermediate groups have a 10-fold increased risk of death (p = 0.03), while the PTLD index does not permit definite stratification of patients into different risk groups (p = 0.08). Nevertheless, according to the PTLD index, patients in intermediate and high-risk groups have a 3-6-fold increased relative risk of death (Table 4).

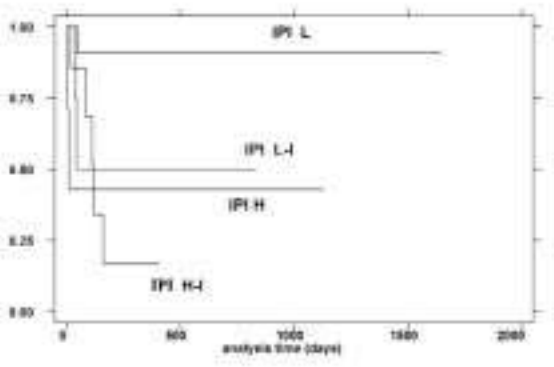

Figure 2. Kaplan-Meier survival estimates according to IPI index: Group L = low risk; Group L-I = low-intermediate risk; Group H-I = high-intermediate risk; Group H = high-risk.

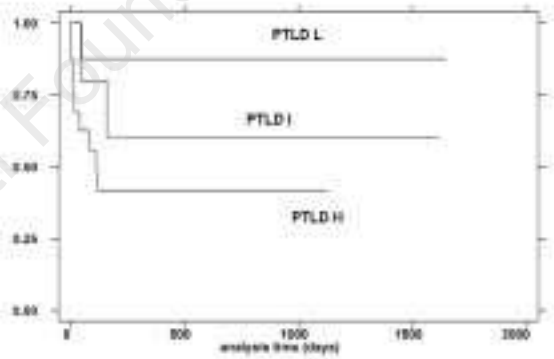

Figure 3. Kaplan-Meier survival estimates according to PTLD index: Group L = low risk; Group I = intermediate risk; Group H = high risk.

Discussion

PTLDs have been identified as distinct clinical and pathologic entities since 1969.[30] (The most relevant aims of recent studies are better assessment of biological and clinical features, and the definition of the most appropriate first-line therapy. In earlier reports EBV-negative forms represented approximately 10% of cases, while recently a higher rate of EBV-negative PTLDs has been reported, along with an increase of late-onset forms.[14,16,31,32] These epidemiological differences might be related to the different intensity over time of immunosuppressive regimens.[16] Moreover, better survival and longer follow-up of transplanted patients increase the risk of late complications, including late-onset PTLDs. The results of our study are in line with those of recent reports:[14,16,31,32] after 1997 the prevalence of late-onset forms is higher than that in earlier years, and the median time from transplant to PTLD has increased. However, in our population, the EBV-negative PTLD prevalence has remained steady (30 and 31%).

Compared to EBV-positive forms, in our series EBV-negative PTLDs occurred later, were all malignant lymphomas, and more frequently presented with limited disease and better performance status. Moreover, in EBV-negative patients lower levels of EBV DNA copies and IL10, both strictly related to the development of PTLD,[33-37] have been detected. The diversity of these clinical and pathologic features might reflect different oncogenetic behaviors, with special reference to the role of EBV in lymphomagenesis. Better knowledge of oncogenetic pathways could have important implications for the management of these patients.

Regarding the prognostic assessment, our study confirms the prognostic value of performance status, clinical stage and LDH, as in immunocompetent patients. Both the IPI[29] and the PTLD index[14] are able to identify different risk groups, but the prognostic ability of IPI is greater than that of the PTLD index (explaned variation 0.26 and 0.16, respectively).

At present, there is no standardized approach to the patient with PTLD.[3,38] Reduction of immuno-

suppression is considered the first step of treatment, and disease resolution is reported in up to 25% of patients.[38,39] In our experience, however, none of the patients responded or showed disease improvement after reduction of immunosuppression alone. Whenever possible, additional treatments had to be given to all patients. We consider decreased immunosuppression alone as the correct first-line intervention only in polymorphic forms (usually EBV-positive). Instead, monomorphic monoclonal forms require prompt standard treatment for lymphomas, as indicated for immunocompetent patients.

In our experience 12 out of 18 patients (67%) entered CR after chemotherapy as first-(11 patients) or second-line treatment (one patient). As recently observed,[40,41] chemotherapy does not seem to be associated with the high mortality rates reported in earlier series.[2,3,9] In our study, in univariate analysis chemotherapy decreased relative risk of death to a third (HR 0.32-CI 0.10-0.99). Extensive supportive care, however, is mandatory and some scheduled treatments have to be modified (either dose reduction or single drug replacement). The efficacy of antiviral agents remains uncertain.[1-3,38] Our study population is too small to permit any definite conclusion: in our experience, one complete response was observed out of three patients (two PH forms and one PLD form) treated with antiviral ± HDIg as first-line therapy, and 6 out of 9 EBV-positive ML patients entered CR with combined chemotherapy + antiviral treatment, without additional toxicity.

Good results are reported with anti-CD20 therapy, both in polymorphic and in monoclonal monomorphic forms.[20,42,43] Our experience on the efficacy of rituximab is limited: in our study population the expression of CD20 on pathologic tissues was less than 60% of tested cases, reducing the extensive use of rituximab. Of the two patients given the monoclonal antibody, one underwent surgery followed by rituximab and achieved CR.

Finally, the single patient treated with autologous CTLs for recurrent, multiple-line treatment resistant disease (EBV-positive ML) obtained a good response without any toxicity: experience in a greater number of patients is needed to evaluate the efficacy of CTLs over a long period.

Overall, in order to obtain disease remission while avoiding life-threatening toxicity, we had to employ a wide spectrum of therapeutic tools: conventional (chemotherapy), innovative (autologous CTL), or unusual for hematologic patients (radiofrequency tissue ablation). In our series the cumulative probability of survival at 1 year is 57% (CI 37.6-73.4) and the median survival time of the entire group has not been reached at 54 months (Figure 3). Careful follow-up of transplanted patients, prompt diagnosis and clinico-pathologically adapted treatment are probably the reasons for the improved outcome of patients. In fact, PTLDs are heterogeneous diseases associated with a high mortality rate, which ranges up to 50-80% during the first year after diagnosis,[38] due to the aggressive clinical course, treatment-related toxicity and, sometimes, delayed diagnosis. Other events frequently arising in transplanted patients, such as opportunistic infections or graft rejection, may be confusing factors which delay diagnosis of PTLD; in our experience timely treatment, before worsening of performance status, might be life-saving. The lower prevalence of post-mortem diagnoses (from 36 to 14%) and early deaths (from 18 to 10%) observed in the last years of our study may reflect the impact of an interdisciplinary study started in 1997, including careful follow-up of transplanted patients, aimed at timely diagnosis of PTLD.

An additional problem in the management of these patients is the definition of the best immunosuppressive regimen to administer after successful treatment of PTLDs. In our study population, three patients developed severe chronic graft rejection shortly after completion of chemotherapy, while on reduced-dose immunosuppression. With better survival of PTLD patients, it will be necessary to design immunosuppressive protocols able to achieve a balance between the risk of graft rejection and that of PTLD recurrence.

Contributions and Acknowledgments

GM: analysis and interpretation of clinico-pathologic data; drafting and final approval of the manuscript; SC: drafting of manuscript; clinical follow-up of patients; PLO: revision of all pathologic specimens; CK: statistical analysis; GG, VR, G D'A: collection of clinical data; PC, FB: biological and virological studies; MM, AMN: clinical follow-up of patients; EM: revision and final approval of the manuscript. MF, FO, GB, EM, ADG, AA, GR, EDJ, GZ, LG, RC, LP, LI, EB, EM, SV, MG: clinicians, pathologists and technicians, all members of the interdisciplinary study group, who made the present study possible with their careful follow-up of transplanted patients.

Disclosures

Conflict of interest: none.

Redundant publication: no substantial overlapping with previous papers.

References

1. Mamzer-Bruneel MF, Bourquelot P, Hermine O, Legendre C, Kreis H. Treatment and prognosis of post-transplant lymphoproliferative disease. Ann Transplant 1997; 2:42-8.
2. Boubenider S, Hiesse C, Goupy C, Kriaa F, Marchand S, Charpentier B. Incidence and consequences of post-transplantation lymphoproliferative disorders. J Nephrol 1997; 10: 136-45.
3. Swinnen LJ. Diagnosis and treatment of transplant-related lymphoma. Ann Oncol 2000; 11 Suppl 1:45-8.
4. Swinnen LJ, Costanzo-Nordin MR, Fisher SG, O'Sullivan EJ, Johnson MR, Heroux AL, et al. Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. N Engl J Med 1990; 323:1723-8.
5. List AF, Greco FA, Vogler LB. Lymphoproliferative diseases in immunocompromised hosts: the role of Epstein-Barr virus. J Clin Oncol 1987; 5:1673-89.
6. Tosato G, Taga K, Angiolillo AL, Sgadari C. Epstein-Barr virus as an agent of haematological disease. Bailliére's Clin Haematol 1995; 8:165-99.
7. Ho M. Risk factors and pathogenesis of post-transplant lymphoproliferative disorders. Transplant Proc 1995; 27 (5 Suppl 1):38-40.
8. Lucas KG, Pollok KE, Emanuel DJ. Post-transplant EBV induced lymphoproliferative disorders. Leuk Lymphoma 1997; 25:1-8.
9. Armitage JM, Kormos RL, Stuart RS, Fricker FJ, Griffith BP, Nalesnik M, et al. Post-transplant lymphoproliferative disease in thoracic organ transplant patients: ten years of cyclosporine-based immunosuppression. J Heart Lung Transplant 1991; 10:877-87.
10. Walker RC, Paya CV, Marshall WF, Strickler JG, Wiesner RH, Velosa JA, et al. Pretransplantation seronegative Epstein-Barr virus status is the primary risk factor for post-transplantation lymphoproliferative disorder in adult heart, lung, and other solid organ transplantations. J Heart Lung Transplant 1995; 14:214-21.
11. Manez R, Breinig MC, Linden P, Wilson J, Torre-Cisneros J, Kusne S, et al. Posttransplant lymphoproliferative disease in primary Epstein-Barr virus infection after liver transplantation: the role of cytomegalovirus disease. J Infect Dis 1997; 176:1462-7.
12. Penn I. Cancers complicating organ transplantation. N Engl J Med 1990; 323:1767-9.
13. Leblond V, Davi F, Charlotte F, Dorent R, Bitker MO, Sutton L, et al. Posttransplant lymphoproliferative disorders not associated with Epstein-Barr virus: a distinct entity? J Clin Oncol 1998; 16:2052-9.
14. Leblond V, Dhedin N, Mamzer Bruneel MF, Choquet S, Hermine O, Porcher R, et al. Identification of prognostic factors in 61 patients with posttransplantation lymphoproliferative disorders. J Clin Oncol 2001; 19:772-8.
15. Penn I. Some problems with posttransplant lymphoproliferative disease. Transplantation 2000; 69:705-6.
16. Nelson BP, Nalesnik MA, Bahler DW, Locker J, Fung JJ, Swerdlow SH. Epstein-Barr virus–negative post-transplant lymphoproliferative disorders: a distinct entity? Am J Surg Pathol 2000; 24:375-85.
17. Chadburn A, Cesarman E, Knowles DM. Molecular pathology of posttransplantation lymphoproliferative disorders. Semin Diagn Pathol 1997; 14:15-26.
18. Davis CL, Wood BL, Sabath DE, Joseph JS, Stehman-Breen C, Broudy VC. Interferon-a treatment of posttransplant lymphoproliferative disorder in recipients of solid organ transplants. Transplantation 1998; 66:1770-9.
19. Haque T, Crawford DH. The role of adoptive immunotherapy in the prevention and treatment of lymphoproliferative disease following transplantation. Br J Haematol 1999; 106:309-16.
20. Benkerrou M, Jais JP, Leblond V, Durandy A, Sutton L, Bordigoni P, et al. Anti-B-cell monoclonal antibody treatment of severe posttransplant B-lymphoproliferative disorder: prognostic factors and long-term outcome. Blood 1998; 92:3137-47.
21. Leblond V, Sutton L, Dorent R, Davi F, Bitker MO, Gabarre J, et al. Lymphoproliferative disorders after organ transplantation: a report of 24 cases observed in a single center. J Clin Oncol 1995; 13:961-8.
22. Chadburn A, Chen JM, Hsu DT, Frizzera G, Cesarman E, Garrett TJ, et al. The morphologic and molecular genetic categories of posttransplantation lymphoproliferative disorders are clinically relevant. Cancer 1998; 15:1978-87.
23. Cesarman E, Chadburn A, Liu YF, Migliazza A, Dalla Favera R, Knowles DM. BCL-6 gene mutations in posttransplantation lymphoproliferative disorders predict response to therapy and clinical outcome. Blood 1998; 92:2294-302.
24. Knowles DM, Cesarman E, Chadburn A, Frizzera G, Chen J, Rose EA, et al. Correlative morphologic and molecular genetic analysis demonstrates three distinct categories of posttransplantation lymphoproliferative disorders. Blood 1995; 85:552-65.
25. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994; 84:1361-92.
26. Baldanti F, Grossi P, Furione M, Simoncini L, Sarasini A, Comoli P, et al. High levels of Epstein-Barr virus DNA in blood of solid-organ transplant recipients and their value in predicting posttransplant lymphoproliferative disorders. J Clin Microbiol 2000; 38:613-9.
27. Swinnen LJ, Mullen GM, Carr TJ, Costanzo MR, Fisher RI. Aggressive treatment for postcardiac transplant lymphoproliferation. Blood 1995; 86:3333-40.
28. Rooney CM, Smith CA, Ng CY, Loftin S, Li C, Krance RA, et al. Use of gene–modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet 1995; 345:9-13.
29. Anonymous. A predictive model for aggressive non Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 1993; 329:987-94.
30. Penn I, Hammond W, Brettschneider L, Starzl TE. Malignant lymphomas in transplantation patients. Transplant Proc 1969, 1:106-12.
31. Harris NL, Ferry JA, Swerdlow SH. Posttransplant lymphoproliferative disorders: summary of Society for Hematopathology Workshop. Semin Diagn Pathol 1997; 14:8-14.
32. Dotti G, Fiocchi R, Motta T, Gamba A, Gotti E, Gridelli B, et al. Epstein-Barr virus-negative lymphoproliferative disorders in long-term survivors after heart, kidney, and liver transplant. Transplantation 2000; 69:827-33.
33. Garnier JL, Blanc-Brunat N, Vivier G, Rousset F, Touraine

JL. Interleukin-10 in Epstein-Barr virus-associated post-transplant lymphomas. Clin Transplant 1999; 13:305-12.
34. Birkeland SA, Bendtzen K, Moller B, Hamilton-Dutoit S, Andersen HK. Interleukin-10 and posttransplant lymphoproliferative disorder after kidney transplantation. Transplantation 1999; 67:876-81.
35. Haque T, Thomas JA, Parratt R, Hunt BJ, Yacoub MH, Crawford DH. A prospective study in heart and lung transplant recipients correlating persistent Epstein-Barr virus infection with clinical events. Transplantation 1997; 64:1028-34.
36. Straus SE, Cohen JI, Tosato G, Meier J. NIH conference. Epstein-Barr virus infections: biology, pathogenesis, and management. Ann Intern Med 1993; 118:45-58.
37. Stevens SJ, Verschuuren EA, Pronk I, van Der Bij W, Harmsen MC, The TH, et al. Frequent monitoring of Epstein-Barr virus DNA load in unfractionated whole blood is essential for early detection of posttransplant lymphoproliferative disease in high-risk patients. Blood 2001; 97:1165-71.
38. Paya CV, Fung JJ, Nalesnik MA, Kieff E, Green M, Gores G, et al. Epstein-Barr virus-induced posttransplant lymphoproliferative disorders. ASTS/ASTP EBV-PTLD Task Force and The Mayo Clinic Organized International Consensus Development Meeting. Transplantation 1999; 68: 1517-25.
39. Starzl TE, Nalesnik MA, Porter KA, Ho M, Iwatsuki S, Griffith BP, et al. Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporin-steroid therapy. Lancet 1984; 1:583-7.
40. Gross TG, Hinrichs SH, Winner J, Greiner TC, Kaufman SS, Sammut PH, et al. Treatment of post-transplant lymphoproliferative disease (PTLD) following solid organ transplantation with low-dose chemotherapy. Ann Oncol 1998; 9:339-40.
41. Mamzer-Bruneel MF, Lome C, Morelon E, Levy V, Bourquelot P, Jacobs F, et al. Durable remission after aggressive chemotherapy for very late post-kidney transplant lymphoproliferation: a report of 16 cases observed in a single center. J Clin Oncol 2000; 18:3622-32.
42. Dotti G, Rambaldi A, Fiocchi R, Motta T, Torre G, Viero P, et al. Anti-CD20 antibody (rituximab) administration in patients with late occurring lymphomas after solid organ transplant. Haematologica 2001; 86:618-23.
43. Oertel SH, Anagnostopoulos I, Bechstein WO, Liehr H, Riess HB. Treatment of posttransplant lymphoproliferative disorder with the anti-CD20 monoclonal antibody rituximab alone in an adult after liver transplantation: a new drug in therapy of patients with posttransplant lymphoproliferative disorder after solid organ transplantation? Transplantation 2000; 69:430-2.

Peer Review Outcomes

What is already known on this topic

Post-transplant lymphoproliferative disorders are less rare than thought, have variable clinical pictures and represent a non negligible cause of mortality in allograft recipient.

What this study adds

The International Prognostic Index (IPI) is currently the best tool for predicting prognosis in patients with post-transplant lymphoproliferative disorders. A risk-adapted therapy may result in long-lasting complete responses and may improve overall survival.

Manuscript processing

This manuscript was peer reviewed by two external referees and by Prof. Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Prof. Cazzola and the Editors. Manuscript received April 12, 2001; accepted October 6, 2001.

Potential implications for clinical practice

This manuscript provides evidence for the effectiveness and low toxicity of therapies for PTLD. With better survival of PTLD patients, the risk of graft rejection following reduction of immunosuppression in responders is highlighted.

Mario Cazzola, Editor-in-Chief