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Post-Transplant Lymphoproliferative Disorders (PTLD) in Liver Transplantation (LTX): A Twenty Year Experience

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Abbreviations: LTX - liver transplantation; EBV – Epstein-Barr Virus; HCV – Hepatitis C Virus; PTLD - post-transplant lymphoproliferative disease; CsA – Cyclosporin A; Tac – Tacrolimus; MMF – mycophenolate mofetil; OKT3 – Orthoclone OKT3

Keywords: Liver transplantation, Immunosuppression, Post-transplant lymphoproliferative disease, Epstein-Barr virus

ABSTRACT

Objective: To evaluate the incidence of PTLD, the risk factors and the impact of this complication on survival outcomes in a large cohort of LTX recipients at a single institution.

Background: LTX has been accepted as a therapeutic option for patients with end-stage liver disease since 1983, in large part due to the availability and reliance on the use of non-specifically directed immunosuppression. However, as predicted and subsequently verified in 1968, an increased incidence of certain de novo malignancies has been observed, particularly with regards to lymphoid neoplasms. While many reports have confirmed and clarified the nature of PTLD, the literature is fraught with conflicting experience and outcomes with PTLD.

Patients and Methods: Four thousand consecutive patients, who underwent LTX between February 1981 and April 1998, were included in this analysis and were followed to November 2001. The effect of recipient age at the time of LTX, recipient gender, diagnosis, baseline immunosuppression, grading of PTLD, and association with Epstein-Barr virus (EBV) were compared. The causes of death were also examined. Treatment for PTLD varied over the 20-year period, but all included massive reduction or elimination of baseline immunosuppression.

Results: The one year patient survival for LTX patients with PTLD is 85%, while the overall patient survival for the entire cohort is 53%. The actuarial 20-year survival is estimated at 45%. The overall median time to PTLD presentation was 10 months and children had an incidence of PTLD that was three-fold higher than adults. Patient survival was better in the pediatric age group, in patients transplanted in the era of Tac immunosuppression, in patients with polymorphic PTLD, and those with limited disease. Interestingly, neither the presence or absence of EBV, nor the timing of PTLD presentation, appeared to influence overall patient survival. LTX patients transplanted for alcohol-related liver disease had similar incidence of PTLD but had a higher risk of mortality.

Conclusion: While PTLD continues to pose problems in LTX, improvements in patient survival have been observed over time. While it is too early to assess the impact of new advances in prophylaxis, diagnosis, and treatment, such approaches are based upon an increased knowledge of the pathophysiology of PTLD.

INTRODUCTION

Transplantation of solid organs has been successful, in large part due to the development of immunosuppressive regimens that have controlled the recipient immune system from rejecting the allograft. By suppressing recipient T lymphocytes with cyclosporin (CsA) or tacrolimus (Tac), or reversing rejection with anti-lymphocyte agents, such as ATGAM or OKT3, rejection has become a rare cause of allograft loss (1). However, the penalty for the non-specific nature of immunosuppression is the susceptibility of the recipient to the development of opportunistic infections (including viral, fungal and protozoal organisms), as well as the increased risk of developing malignancies (2).

Post-transplant lymphoproliferative disease (PTLD) can be considered, for the most part, an opportunistic infectious complication that arises after transplantation, usually involving the Epstein-Barr virus (EBV) (3). Lymphoid tumors were first described in transplant patients in 1968 and were called "reticulum cell sarcomas"; a subgroup of these was termed "pseudolymphomas" in recognition of their ability to undergo regression after reduction of immunosuppression (4-6). PTLD describes a heterogeneous group of lymphoproliferative diseases ranging from benign polyclonal B cell proliferation, as seen in acute EBV infections (e.g. mononucleosis), to a relatively malignant monoclonal lymphomatous lesion. In addition, the spectrum of presentations varies from localized to disseminated involvement, and nodal to extranodal, including the allograft organ itself (7, 8).

The risk factors and incidence of PTLD, as well as outcomes after the development of this complication in LTX are not clearly appreciated, in part due to variations in the study population, changing definitions of PTLD, improved detection methods and higher index of suspicion. The current study will assess the incidence of PTLD, the risk factors and the impact of this complication on survival outcomes in a large cohort of LTX recipients at a single institution.

MATERIALS AND METHODS

The study subjects are first 4000 consecutive patients who underwent LTX since the inception of program at the University of Pittsburgh starting in February 1981 to April 1998, and have been described elsewhere (1). Briefly, this group of patients received a total of 4947 allografts. Nine hundred and twenty two patients in our overall LTX experience were excluded from analysis, either because they were transplanted at the VA Medical Center, received combined liver and intestinal allografts, or did not have a minimum of three years follow-up. The mean follow-up was 11.8 $\pm$ 3.9 years (median 11.7: range 3 to 20 years). There were 2172 (54.3%) males and 1828 (45.7%) females. The study populations were analyzed based on the age of the recipient at the time of transplant (i.e. adult vs. pediatric), and into two timeframes (based on the routine use of CsA or Tac). The demographics of the patients studied, with respect to immunosuppression, age, and follow-up time are shown in Table 1. The follow-up between patients on CsA was significantly longer than for Tac ($p<0.01$).

A single experienced transplant pathologist (MN), in a blinded fashion, reviewed all PTLD specimens that were available from the beginning of the program. Since 1991, in situ hybridization with probes to detect the EBER-1 gene was added to document the presence or absence of EBV in PTLD specimens (9). The grading scoring system used was adapted from the Society for Hematopathology Workshop held in 1995, which defined the spectrum of recognized PTLD (10). A summary of the characteristics used define reactive or early lesions, polymorphic PTLD, and lesions which appeared to represent lymphomas or hematopoietic neoplasms, including a category for lesions such as plasmacytomas, T cell rich B-cell lymphomas, and T cell lymphomas under the umbrella term of PTLD, is shown in Table 2.

All patient information was collected prospectively and entered into the Thomas Starzl Transplantation Institute Electronic Data Interface for Transplantation (EDIT) which store demographics, laboratory tests, medications, pathology, and other relevant clinical information by interfacing with all hospital information systems and also include manually entered data from external sources. Data for analysis was rendered anonymous by stripping it of unique patient

identifiers by an "honest broker", according to the requirements of the exempt University of Pittsburgh Institutional Review Board approved protocol (IRB#020177).

Statistical Analysis

Kaplan-Meier estimates were used to calculate survival curves. Differences in survival curves were compared using log rank statistics. Differences in proportions were tested using chi square test (or Fisher exact test). "P" values less than 0.05 were considered statistically significant.

RESULTS

Incidence of PTLD

Of the 4000 LTX patients studied, a total of 170 patients (4.3%) were found to have PTLD, 10 of which were only diagnosed or confirmed at the time of autopsy (11). The incidence of PTLD was significantly higher in children (9.7%) as compared to adults (2.9%) ($p<0.01$). Although the overall incidence of PTLD was similar between CsA and Tac treated patients, the incidence of PTLD in the pediatric group was higher under Tac than CsA (12.6% vs. 7.7%, $p=0.06$), while the incidence of PTLD in adults was no different based on immunosuppression (3.1% CsA vs. 2.6% Tac). The difference in pediatric PTLD incidence is in part due to a significantly higher early mortality in the CsA group vs. the Tac group (32% vs 15% at three years post-LTX, respectively), thus reducing the at-risk population (12). The diagnosis-associated incidence of PTLD is shown in Table 3. The higher rate of PTLD in the "Metabolic" and "Biliary Atresia" group reflects the preponderance of children for these two indications.

Timing of PTLD

The overall median time to development of PTLD was 10 months - this was significantly shorter in children compared to adults (8.1 months vs. 15 months, $p=0.02$). Within the adult population, CsA was associated with a shorter time to PTLD development compared to Tac (6.1 months vs. 23.6 months, $p=0.01$), while the opposite was true for the pediatric population (27 months CsA vs. 4.7 months Tac). Figure 1 demonstrates the wide range in the timing to developing PTLD.

Presentation of PTLD

In hematologic malignancies, staging of disease correlates with treatment response and survival, but little data exists in the area of PTLD. Table 4 summarizes the number of patients with single or multiple site involvement with PTLD. A slightly greater number of patients presented with single site involvement (58%) compared to multiple sites (41%) (p=n.s.) and this was no different amongst the age or immunosuppressive categories. The locations of PTLD are shown in Table 5. Lymph nodes were the most predominant site involved (35%), in children this was higher (41%) compared to adults (31%). Gastrointestinal involvement was seen in 25%, while the liver and spleen was involved in 16% of cases. CNS involvement was present in only 4% of cases.

Grading of PTLD

Using the grading system adopted by the Society for Hematopathology and revised by European-American Lymphoma Group (10), 148 of the 170 PTLD were classified. As shown in Table 6, 12% were considered as "Early Lesions" Grade I, 35% were classified as "PTLD – Polymorphic" Grade II, 43% were categorized as "PTLD – Monomorphic" Grade III, and 10% were classified as "PTLD – Other" Grade IV. While there were no notable differences in the grading based on type of immunosuppression, there was a notable difference between adults and children. Sixty-eight percent of pediatric PTLD were classified as Grades I or II, while 70% of adult PTLD were classified as Grades III or IV ($p<0.01$). Two cases in Grade IV were T-cell PTLD.

Role of EBV

With the availability of probes to detect the EBV-encoded small RNA, EBV can be detected in paraffin-fixed specimens using in situ hybridization (9). EBER was positive in 80% of samples, while EBER was not detected in 20% of the 104 PTLD samples studied. Of interest was that 98% of pediatric PTLD were EBER positive, while only 68% of adult PTLD were EBER positive.

Patient Survival

The actuarial patient survival for entire population of PTLD patients at 1, 5, 10, 15 and 20 years was 85, 69, 55, 47 and 45 percent respectively, as shown in Figure 2. While there was numerical difference in survival with women having a better survival than men, this was only evident at 10 years after PTLD diagnosis and did not reach statistical significance. Long-term survival rates for pediatric patients with PTLD were better than for adults (60% pediatric at 15 years, compared to 39% for adults), but did not quite reach significant difference ($p=0.06$) (Figure 3). As a reflection of both the impact of improvements over time and with immunosuppression, survival in the Tac group was significantly better than for CsA (60% for Tac, vs. 40% for CsA by 12 years) ($p<0.02$) (Figure 4). Other factors that appeared to have a positive effect on survival included: Grade I PTLD vs. Grades II-IV PTLD ($p=0.04$) (Figure 5), and single site vs. multiple site ($p<0.02$) (Figure 6). No effect of EBER positivity, or time to development of PTLD was apparent on patient survival (data not shown). As noted in Table 3, patients transplanted for alcohol related liver disease, who developed PTLD, had a higher risk of dying.

Causes of Death

A total of 80 patients (47.1%) died during the entire follow-up period. The causes of death are shown in Table 7. PTLD was thought to be the major contributing cause of death in 44% (n=35) patients with PTLD. Infection and multisystem organ failure accounted for the second most common causes of death, comprising 28% of all deaths. This was followed by recurrent or de novo cancers (5%) and recurrent disease (5%).

Treatment

As one would expect, treatment for PTLD varied considerably over the twenty-year period. However, the mainstay of treatment was immunosuppressive drug reduction or discontinuation in all cases where this could be documented (the exceptions are those patients in whom the diagnosis of PTLD was not made ante mortem). Other treatments included: antiviral therapy, e.g. acyclovir or ganciclovir, in 82/122 cases (67%), followed by chemotherapy or radiotherapy (31%), surgery (17%) and more recently, the use of anti-B cell monoclonal antibodies (11%). Immunomodulation or immunotherapy was attempted in 6% of cases.

DISCUSSION

The overall incidence of PTLD in LTX patients is estimated to occur at 2-3% overall (2, 8, 13, 14). However, there are populations of LTX recipients, which can be identified as "high-risk" patients. These include: lack of previous EBV infection (i.e. EBV seronegative); pediatric transplant recipients; and those that receive anti-lymphocyte antibodies. These risk factors are at least additive, so that in the pediatric LTX transplant recipient, who is EBV seronegative, and requires anti-lymphocyte antibodies is extremely high-risk (PTLD risk up to 30%) (8, 15-17). Other risk factors for PTLD have been implicated; such as HCV co-infection in liver transplant recipients (18, 19). We did not observe this correlation in our patients – the incidence of PTLD in the HCV+ group was 3.01% vs. 2.85% of HCV- adult LTX recipients.

Prior to 1981, lymphoid tumors in transplant patients were uniformly referred to as immunoblastic sarcomas. That year, Frizzera and colleagues from the University of Minnesota examined tumors from a small number of renal transplant recipients (20). They observed several forms of lymphoproliferation, which had not been previously described and applied the term "polymorphic" to emphasize the heterogeneity in size and shape of the tumor cells. Ancillary studies showed the tumors to be comprised of B-lymphocytes. They also stressed that the behavior of PTLD could not be reliably predicted by pathologic studies alone. In 1988, we reported our experience with PTLD observed in the University of Pittsburgh transplant population. We were unable to discern any significant difference in the clinical behavior of the two types of polymorphic lesions and combined them under the heading of polymorphic PTLD. In contrast, lesions, which resembled typical non-Hodgkin's lymphomas, were recognized as a variant of PTLD and the term, monomorphic PTLD, was introduced to distinguish these lesions from polymorphic PTLD (7). As shown in this study, we were unable to show an association between poorer outcomes and more advanced grades of PTLD, although the "early" lesions appeared to have a better survival rate. However, it has been suggested that further categorization of PTLD is possible based on combined pathologic and molecular features – specifically, that more recalcitrant tumors have a monomorphic histology, are monoclonal, and contain rearrangements of the c-myc proto-oncogene (21). We did not routinely perform analysis for chromosomal rearrangements in our experience, however others and we have clearly

shown that gene analysis can help to assess clonality and possibly prognosis (21-24). Broader application of molecular techniques may help to distinguish lower risk from higher risk PTLD for purposes of treatment.

Treatment of PTLD is one of the most controversial areas in solid organ transplantation (12). The lack of a clear consensus in the management of patients with established PTLD stems, is in part, a result of the limited understanding of its pathogenesis, the lack of characterization of PTLD, and the specific immune defects associated with PTLD. Nevertheless, based on single center reports, four major areas of treatment should be considered: a) reduction of immunosuppression; b) chemo- and biologic therapy; c) anti-B cell monoclonal antibody therapy; and d) cell based therapies. We believe that the comparatively good outcomes for PTLD reported here, and that span the 20 year existence of this program hinges on the principle of recovery of the recipients immune response leading to modulation of the PTLD, generally by reducing or eliminating immunosuppression (6). Our current algorithm for treatment in documented or suspected PTLD is the initial intervention of reduction in immunosuppression. However, how much reduction, for how long, and how to predict the response to such reduction is unknown. Regression of monoclonal and polyclonal lesions following reduction of the dose of immunosuppression ranges from 23% to 50% (6, 12). Potential consideration for the level of immunosuppression reduction should assess the following: the severity of illness, the location and presentation of PTLD, the length and type of immunosuppressive therapy, and the immunohistochemical and molecular characterization of the PTLD. At the ASTP/ASTS Workshop on PTLD (12), the consensus in critically ill patients with extensive disease is to decrease prednisone to a maintenance dose of 7.5-10 mg/day and all other immunosuppression stopped. If there is no response or the response is not adequate within 7-21 days, then more aggressive interventions should be considered. In addition, in the less critically ill patient with limited disease, the initial management strategy should include reduction of CsA or Tac and prednisone by at least 50%, while azathioprine or MMF should be discontinued. After a 14-day trial of decreased immunosuppression, a further decrease of 25% can be considered. Should clinical urgency or failure of "conservative" therapy develop, chemotherapy using a lymphoma protocol has generally been adopted (12, 25-27). Alternatively, promising results using anti-B cell monoclonal antibodies have been reported, beginning with the initial results of Fischer and

coworkers using a combination of anti-CD21 and anti-CD24 monoclonal antibodies (28). The availability of anti-CD20 monoclonal antibody, which targets most B-cells, has been shown to have promising preliminary results (29, 30).

The results of this approach reveal that short-term and long-term patient survival were not as dismal as reported in other series (14, 26). Nevertheless, the decline in survival was approximately 6% per year in the adult PTLD population and 4% per year in the pediatric PTLD population, worse than that observed in the LTX group as a whole (approximately 3.5% and 1.5% per year decline, respectively) (1). The fact that PTLD contributed to 44% of deaths in patients afflicted with PTLD highlights the need for advances in prophylaxis, detection, treatment of PTLD, as well as a better understanding of other risk factors associated with this disease. We also note that while LTX patients transplanted for alcoholic liver disease had similar risks for PTLD, their long-term mortality rate was significantly higher (87%) compared to 50% in non-alcoholic LTX patients. It may be that the reported karotypic chromosomal lymphocyte aberrations associated with ethanol (31) may potentiate the known effect of EBV in causing chromosomal translocation (reviewed in 8).

Green and colleagues at the University of Pittsburgh have initiated a study examining the use of high titer anti-EBV intravenous immunoglobulin for EBV-associated PTLD in high-risk pediatric recipients (32). McDiarmid and others from UCLA have suggested that the use of intravenous ganciclovir in high-risk EBV+ donors to EBV- recipients may prevent subsequent PTLD (33).

Rowe and coworkers examined the utility of EBV viral loads as a means to monitor patients at risk for PTLD (34, 35). Others have also suggested that EBV monitoring can be useful in high-risk transplant recipients (36). The Montpellier group has suggested that detection of persistent monoclonal immunoglobulins in LTX recipients was associated with a 23% incidence of PTLD (37). Certainly, if PTLD can be detected preemptively or while still in an "early" stage, the outcomes would likely be better.

Cell based immunotherapy holds promise for the treatment of PTLD, however the use of adoptive cellular immunotherapy using IL-2-stimulated LAK (lymphokine activated killer) cells was associated with a response rate of only 30% in refractory PTLD (38). Targeted approaches using EBV-specific T effector cells have been used in patients with PTLD following bone marrow transplantation since, in these patients, although the PTLD arises from donor B-cells, the EBV-specific effector T cells can be obtained from the donor, who is not under the effect of immunosuppression (39). Similar approaches to generate EBV-specific cytolytic T cells (CTL) in high risk EBV seropositive transplant recipients has also shown promise as a means to preemptively treat patients with elevated EBV viral load (40). In the case of an EBV seronegative solid organ transplant recipient, there are significant technical limitations in priming ex vivo EBV-specific CTL and may limit their expansion and subsequent clinical use. However, recent advances in understanding the biology of antigen presenting cells and especially that growth factors may make this approach possible in the future for treating solid organ transplant recipients (41).

There is increasing recognition that EBV-negative PTLD is a separate entity – these tumors tend to arise later in the post-transplant course (42, 43), and have been reported to have a higher frequency of c-myc rearrangements (44). Although we have seen an increasing detection of EBV negative PTLD (45), as noted in this study, we did not detect any impact on clinical outcomes, recognizing that this entity appears to be mostly restricted to adult transplant recipients. Thus far, no etiologic agent has been found.

Table 1: Patient Demographics

Median Follow-up:	11.7 years
Mean Follow-up:	11.8 ± 3.9 years
Mean Follow-up CsA:	15.2 ± 2.23 years
Mean Follow-up Tac:	8.9 ± 2.5 years
Total patients:	4,000
Males:	2,172 (54%)
Females:	1,828 (46%)
Total CsA:	1,830 (46%)
Total Tac:	2,170 (54%)
Children (Age <18 yrs):	808 (20%)
Children CsA:	482
Children Tac:	326
Adults (Age >18 yrs):	3,192 (80%)
Adult CsA:	1348
Adult Tac:	1844

Table 2: Classification system for PTLD*

Grading	Description
0	EBV lymphoadenititis, hepatitis, not classified as PTLD
1	Early lesion, low-grade mononucleosis, plasma cell hyperplasia
2	Polymorphic, diffuse B-cell hyperplasia (PDBH) and polymorphic B-cell lymphoma (PBC)
3	Monomorphic or lymphomatous PTLD or lymphoma, Immunoblastic lymphoma (IBL), diffuse large cell B-cell or diffuse small cell noncleaved (Burkitt-like)
4	Other Hodgkins-like PTLD, plasma cell lesions, plasmacytoma, T cell PTLD

* Adapted from Harris et al (10)

Table 3: Incidence of PTLD Based on Grouping for LTX Indications

Diagnosis	Cases	no. of PTLD	% of PTLD	% survival
Alcoholic	567	16	2.8	12.5
HCV+NANB	655	20	3.1	50
Malignancy	301	5	1.7	40
PBC+PSC+AI	829	31	3.7	64.5
HBV	219	3	1.4	66.7
Metabolic	227	17	7.5	41.2
Biliary Atresia	429	37	8.6	59.5
Fulminant Failure	124	7	5.6	71.40
Other	649	34	5.2	52.9
Total	**4000**	**170**	**4.3**	**52.9**

Table 4: Extent of PTLD Involvement – Single vs. Multiple Sites

	Single	Multiple	Total Sites	PTLD per Pt.
Adults	52	40 (97)	149	1.62
CsA	28	18		
Tac	24	22		
Children	48	30 (75)	123	1.58
CsA	24	13		
Tac	24	17		
All CsA	52	31		
All Tac	48	39		
Overall	100 (58.8%)	70 (41.2%)	272	1.6

*Tonsil and/or adenoids and/or cervical lymph node in any combination or alone, GI single or multiple lesions with or without mesenteric nodes were considered as one site.

Table 5: Location of PTLD Involvement

	Children			Adults			Total
	CsA	Tac	Total Children	CsA	Tac	Total Adult	n (%)
Lymph node	26	24	50	23	23	46	96 (35.2)
GI	10	18	28	17	22	39	67 (24.6)
Liver, Spleen	10	15	25	11	8	19	44 (16.2)
Pulmonary	8	2	10	8	11	19	29 (10.7)
CNS	3	2	5	1	5	6	11 (4.0)
Other	4	1	5	9	11	20	25 (9.2)

Table 6: Grading of PTLD*

Grade	CyA n (%)	Tac n (%)	Children n (%)	Adults n (%)	Total n (%)
I	7 (10)	11 (14)	12 (17)	6 (8)	18 (12)
II	21 (31)	31 (39)	35 (51)	17 (22)	52 (35)
III	32 (47)	31 (39)	20 (29)	43 (54)	63 (43)
IV	8 (12)	7 (9)	2 (3)	13 (16)	15 (10)
Total	68	80	69	79	148

*Using criteria described by Harris et al (10) – a total of 148 cases available.

Table 7: Causes of Death

Causes	Number	%
PTLD	35	44
Infection	18	23
Multisystem Organ Failure	4	5
Recurrent disease, Graft Failure	4	5
Recurrent/De Novo Malignancy	3	4
Gastrointestinal	3	4
Cardiac System	3	4
Respiratory System	2	2
Trauma, motor vehicle accident	1	1
Intra cranial bleed	1	1
Unknown causes	6	7
Total	80	

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Figure 1: Incidence of PTLD as a measure of time after LTX. Y-axis represents actual number of cases.

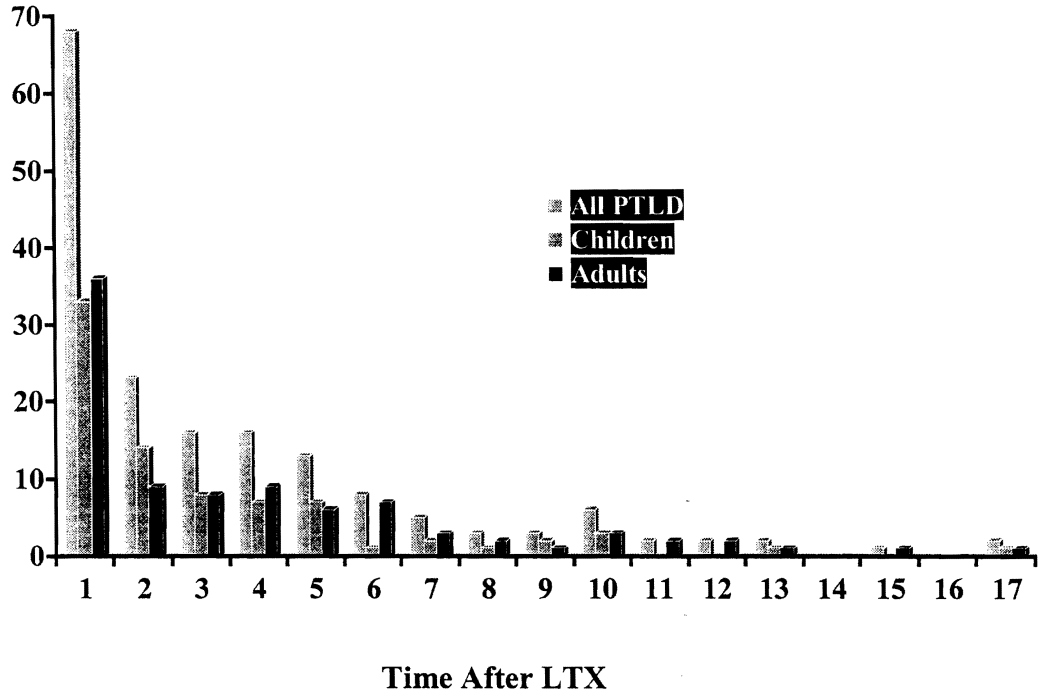

Figure 2: Overall Survival After PTLD Diagnosis

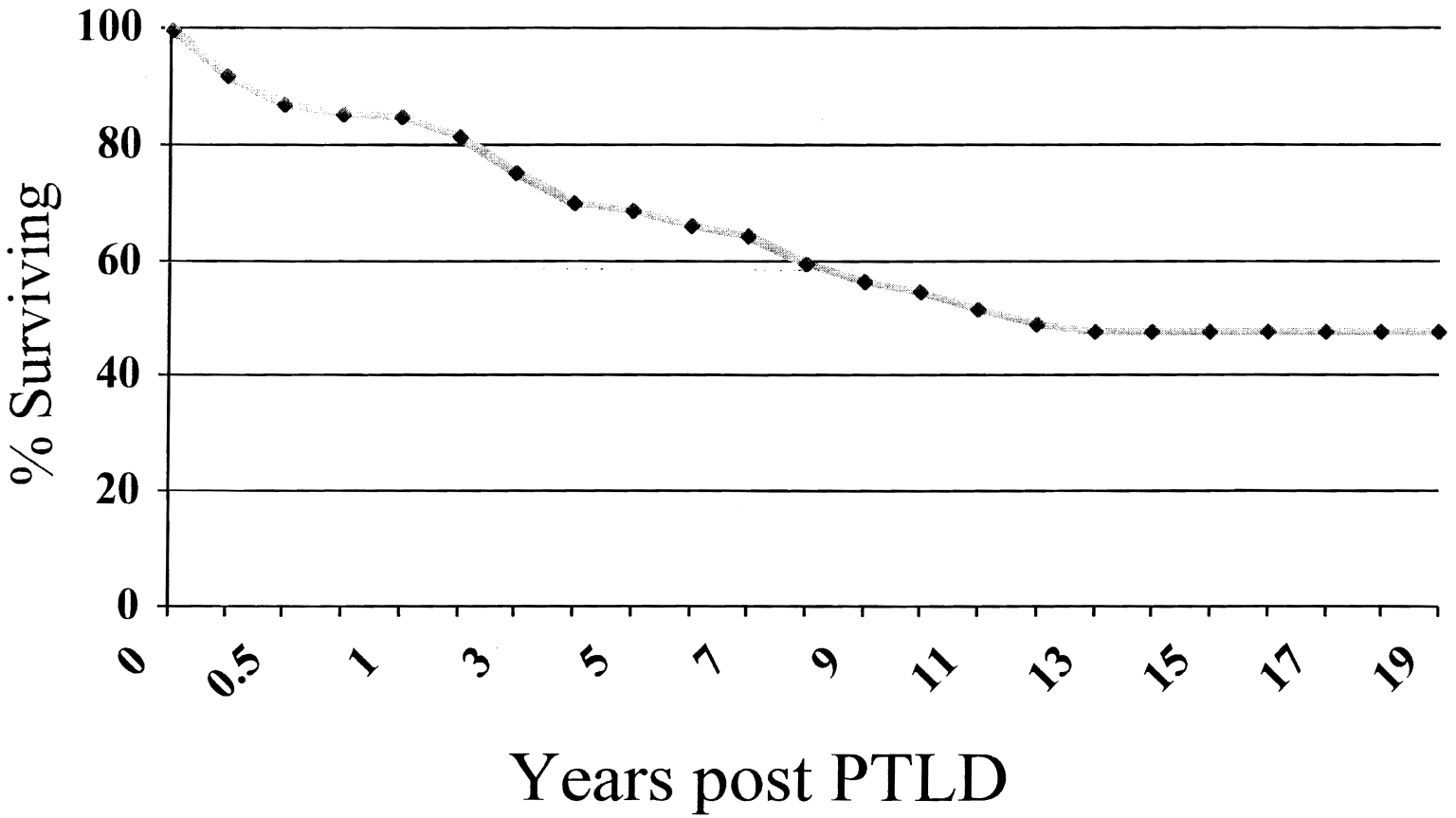

Figure 3: Survival After PTLD Diagnosis - Age Effect

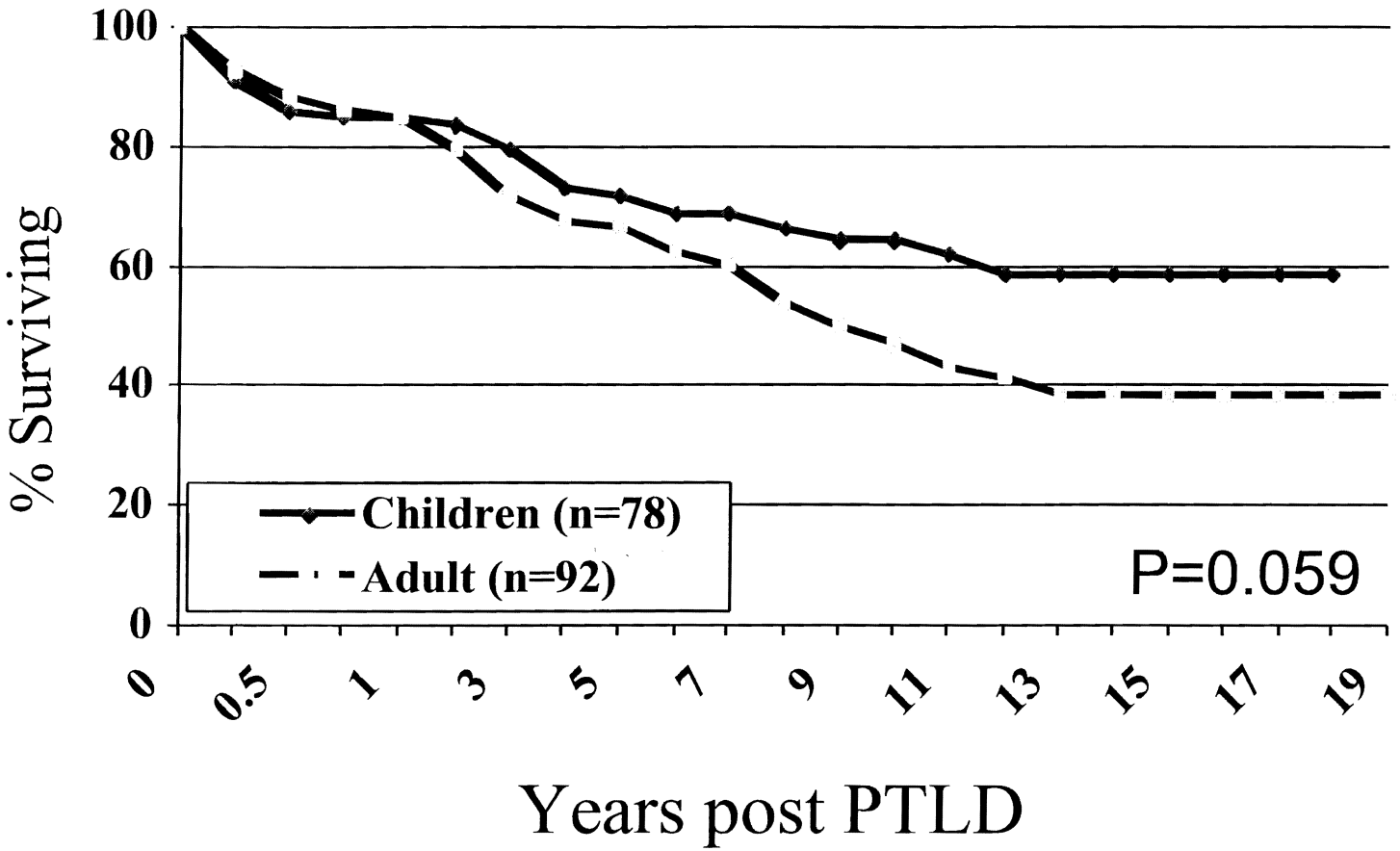

Figure 4: Survival After PTLD Diagnosis - Effect of Baseline Immunosuppressive Agent

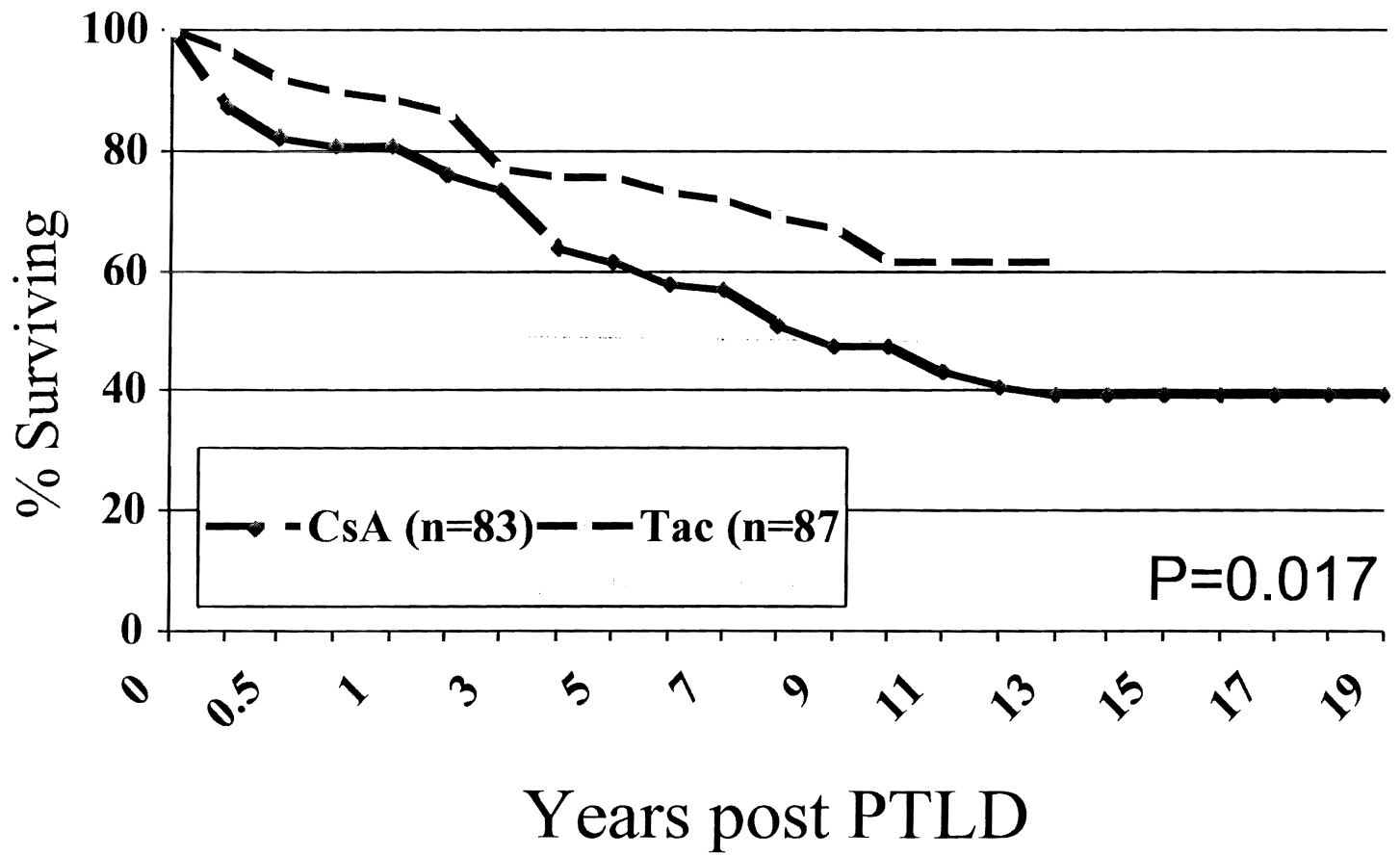

Figure 5: Survival After PTLD Diagnosis - Effect of Grade

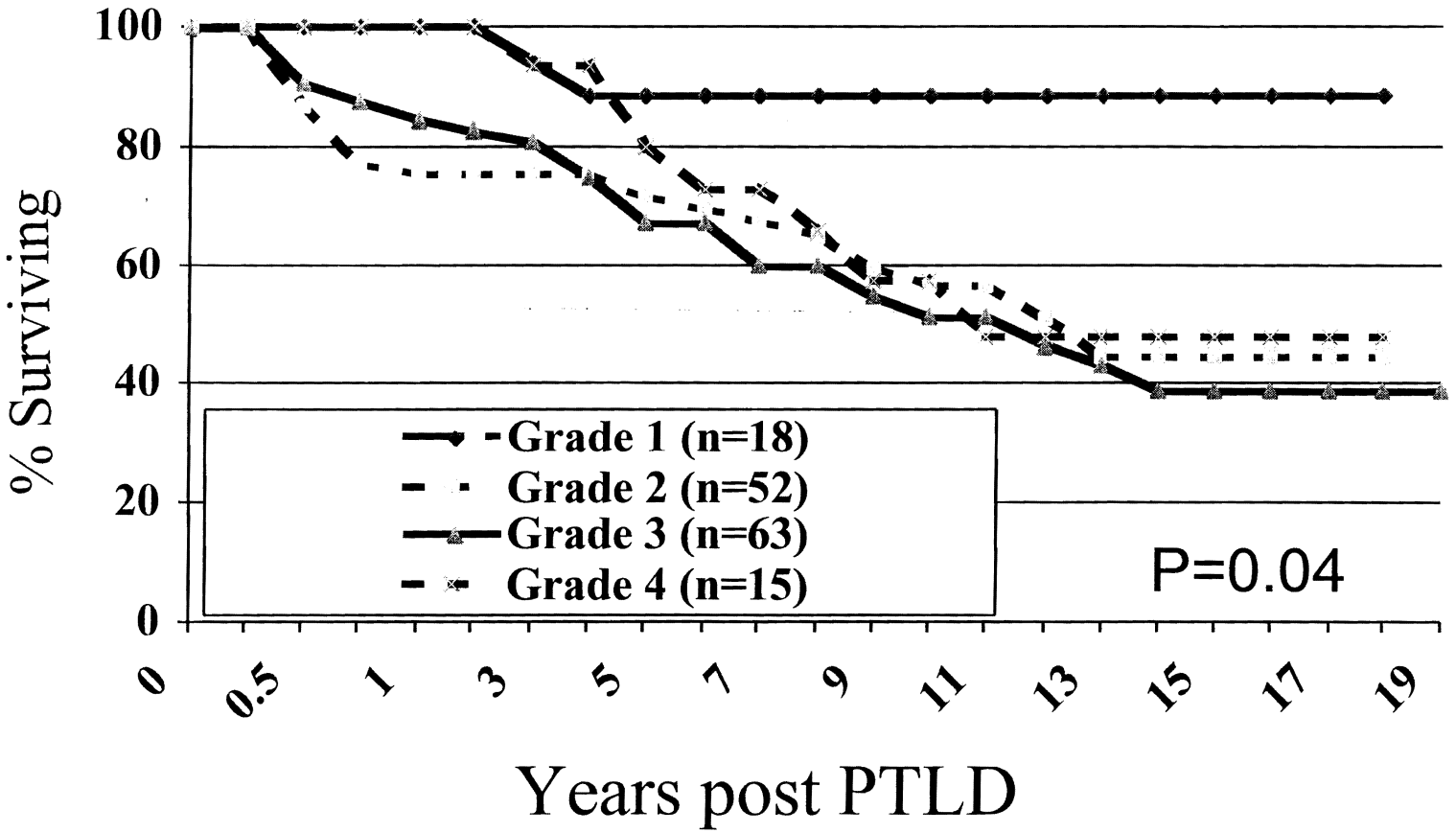

Figure 6: Survival After PTLD Diagnosis - Effect of Disease Dissemination