Published in final edited form as:

J Pediatr Hematol Oncol. 2008 September ; 30(9): 684–688. doi:10.1097/MPH.0b013e31817eb7ca.

BCL6 Expression Correlates with Monomorphic Histology in Children with Post-Transplantation Lymphoproliferative Disease

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Abstract

Post-transplantation lymphoproliferative disease (PTLD) is a complication of organ transplantation with high mortality. Predicting response to first line therapy, reduction of immune suppression (RISM), is difficult because of the heterogeneity of lesions and disease behavior. We sought to determine if BCL6 protein expression in PTLD cells is associated with poor outcome. In a cohort of 25 children with PTLD, 9 of the patients' tumor specimens were positive for BCL6 protein expression. Eight of 13 monomorphic lesions were BCL6 positive, compared to 1 of 11 evaluable polymorphic lesions ($p=0.01$). Only 1 of the patients with BCL6 expression responded to reduced immune suppression ($p=0.19$). Recipients of heart transplants who developed PTLD had reduced overall survival rates compared to recipients of other organ transplants that developed PTLD ($p=0.04$).

Keywords

Post-transplant Lymphoproliferative Disease; PTLD; BCL6; prognosis; children

INTRODUCTION

PTLD is a complication of immune suppression after organ transplantation. The incidence is higher in children than adults, ranging from 2.6–9% for kidney, 6.8–13.1% for liver, 10% for heart and 3.8–11.7% for thoracic organ transplants (1). Estimates of mortality from PTLD vary widely, but have been reported to be 50–80% (2).

PTLDs are classified as early lesions, polymorphic PTLD, monomorphic PTLD (including B- and T-cell lymphomas), and other rare types (3). Polymorphic lesions do not appear histologically to be malignant, but may behave as such and may not resolve with reduction of immunosuppressive medications (RISM). Monomorphic lesions are more malignant in appearance, but may behave less aggressively than typical lymphomas and may resolve with RISM alone or with low doses of chemotherapy (4). Lesions can be polyclonal, oligoclonal

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or monoclonal and have been shown to variably contain alterations in oncogenes or tumor suppressor genes (5).

Developing a uniform staging and treatment strategy for PTLT has been difficult because of the heterogeneity of the process and its responses to therapy. The well-established first line of therapy is RISM, to allow the host EBV-specific cytotoxic T-cells to control lymphoproliferation. This is effective in 20–50% of patients with both polyclonal and monoclonal disease (2). While those with monomorphic disease are less likely to respond to RISM (6), the ability to accurately predict which patients will need more aggressive therapy has not been well established.

BCL6 is a proto-oncogene that encodes a transcriptional repressor protein that regulates a broad spectrum of target genes. *BCL6* protein suppresses p53 expression in germinal center B cells (7) and promotes lymphoma development in transgenic mice (8). It is expressed at high levels in germinal center B cells and lymphomas with germinal center B cell phenotypes (9,10). *BCL6* deregulation has been demonstrated to have prognostic importance in large B-cell lymphomas and follicular lymphomas (11,12) and to predict response to rituximab (13). *BCL6* somatic mutations have also been demonstrated in polymorphic PTLT and post-transplantation lymphomas and were predictive of poor response to RISM in those patients with mutations (14).

To our knowledge, the frequency of *BCL6* protein expression in pediatric PTLT relative to clinical outcomes has not been previously reported. We hypothesized that *BCL6* expression in pediatric PTLT is a marker for more transformed, truly malignant disease that is less likely to respond to RISM than *BCL6* negative PTLT. The purpose of this pilot study was to determine the frequency of *BCL6* protein expression in lesions of children with PTLT and to determine if *BCL6* expression was a predictor of response to RISM, relapse free survival (RFS), or overall survival (OS).

MATERIALS AND METHODS

Study Population

Patients diagnosed with PTLT at The Children's Hospital (TCH) between 1992 and 2005 were identified through communication with transplant coordinators, review of cases of PTLT treated through the oncology division, and review of pathologic diagnoses of PTLT. This study was approved by the Colorado Multiple Institutional Review Board.

Evaluations

Medical records of these patients were abstracted for clinical data. Archived, paraffin embedded specimens of lesions obtained at the time of diagnosis of PTLT were evaluated for the presence of *BCL6* protein expression by immunostaining using a monoclonal anti-*BCL6* antibody (DakoCytomation, Carpinteria, CA) and *BCL6* gene rearrangement by fluorescent in situ hybridization (FISH) using the *BCL6* Dual color, Break Apart Rearrangement Probe (Vysis, Inc. Downers Grove, IL). Diagnoses were reclassified according to the current World Health Organization histologic classification scheme (15).

The primary outcome measure was the relationship between *BCL6* expression and response to RISM. A patient's lesion was considered positive for *BCL6* expression if *BCL6* was detected by immunohistochemistry (IHC) in >40% of cells from any biopsy. Those subjects who had resolution of the manifestations of PTLT with RISM and did not require further therapy with low dose chemotherapy or conventional chemotherapy were considered to have responded to RISM.

Secondary outcome measures included the relationships between BCL6 expression and 1) RFS, defined as time to the second pathologic diagnosis of PTLT from the first diagnosis of PTLT, 2) OS, defined as time before death of any cause, measured from the date of diagnosis of PTLT, and 3) histologic subtype of PTLT (monomorphic or polymorphic) as defined by the World Health Organization (15).

Statistical Considerations

Statistical analysis was performed using SAS software (Cary, NC). Response to RISM with regard to BCL6 expression was analyzed with Fisher's exact test, as was the relationship between BCL6 expression and histologic subtype of PTLT. RFS and OS were analyzed using Kaplan-Meier estimates of survival and compared with a logrank test. Cox's proportional hazards regression model was used to assess the influence of BCL6 expression, age, gender, type of transplant and histology on RFS and OS.

RESULTS

Patients and Outcomes

Between 1992 and 2005, 647 organ transplants (268 heart, 154 HSC, 111 liver, 114 kidney) were performed at TCH. During that same period, 25 children were diagnosed with 26 cases of PTLT. Eleven patients had liver transplants (9.9% of liver transplants), 8 heart transplants (3.0%), 3 HSC transplants (1.9%), and 3 kidney transplants (2.6%).

Clinical and morphological data is provided in Table I. Overall mortality was 48.0% (12/25). Four patients had a sepsis-like clinical presentation, all of whom died (pts. 3, 4, 11, 12). Four patients died with active disease (pts 10, 13, 21, 24). One patient with no evidence of disease (NED) died from complications of graft vs. host disease (pt. 19). One patient died from complications of OKT3 treatment for cardiac rejection; his disease was regressing at the time of death (pt. 14). Two patients died from cardiac rejection with NED (pt. 16 & 25). Five of the 13 surviving solid organ transplant recipients had evidence of rejection, but all were managed successfully with immune suppression. Both of the surviving recipients of HSC transplants had mild GVHD managed with immune suppression. No patient underwent re-transplantation.

Reduced immunosuppression was effective therapy for PTLT in 7 of 26 cases (26.9%). Only 2 patients of 13 (15.3%) with monomorphic disease responded to RISM, as compared to 5 of 13 with polymorphic disease ($p=0.38$). One patient (#9) was diagnosed with monomorphic PTLT, diffuse large B-cell lymphoma. He was treated with RISM and had resolution of PTLT. Seven years later he was diagnosed with PTLT, Classical Hodgkin's lymphoma, at which time he was treated with chemotherapy (16). Each of his cases was considered separately in statistical analyses.

BCL6 Analysis

Specimens were evaluable for BCL6 protein expression by IHC from 24 of 26 cases. In 9 of these cases, lesions were positive for BCL6 expression (Figure 1). One of these was patient 9, whose initial lesion (LBCL) was negative, but whose lesion at second diagnosis of PTLT (Hodgkin's lymphoma) was BCL6 positive. FISH analysis was performed in 16 patients' samples. Rearrangement of *BCL6* was not detected in any of the specimens tested, and disomic copy number was present in each.

Eight of 13 monomorphic lesions were BCL6 positive, compared to 1 of 11 evaluable polymorphic lesions (Table II, $p=0.01$). Of the 9 cases positive for BCL6 expression, only 1 (pt 25) responded to RISM, while 6 of 15 cases without BCL6 expression responded to

RISM ($p=0.19$). BCL6 expression did not predict OS or RFS, even if patients with sepsis like presentations were excluded (not shown). Recipients of heart transplants had a significant difference in OS as compared to recipients of other organ transplants ($p=0.04$; not shown). Nine patients had more than one biopsy that were evaluable for BCL6 expression. In four of these patients (pts 4, 9, 15, 16) the presence of BCL6 expression was discrepant between samples. Only one of these had polymorphic disease (pt 4) which did not respond to RISM.

Including the variables BCL6 expression, age <5 years versus age ≥ 5 years, gender, monomorphic versus polymorphic histology, and transplant type in the survival analysis model, no variable was a significant prognostic factor for response to RISM, overall survival or relapse free survival. However, when only BCL6 expression, histology type, and heart transplant vs. other transplant were included in the model, heart transplantation was a significant prognostic factor ($p=0.03$).

DISCUSSION

PTLD is a devastating complication of organ transplantation, occurring in 3.9% of transplant recipients in this cohort between 1992 and 2005, with a mortality rate of 48%, similar to previous reports (1,2,14). Only 27% of patients responded to RISM, as we defined it. Specimens from 9 of 24 evaluated cases were positive for BCL6 expression. BCL6 expression was found significantly more frequently in monomorphic lesions as compared to polymorphic lesions. While not statistically significant, it is noteworthy that only one of the patients with BCL6 expression responded to RISM. However, BCL6 expression did not predict RFS or OS. The only variable that was significantly associated with poor OS was being the recipient of a heart transplant as compared to other organ transplants.

Management of PTLD requires close collaboration among surgeons, transplant physicians, pathologists and oncologists. The determination of when to intervene with more aggressive therapy than simple reduction of immune suppression can be difficult, particularly when the threat of organ loss due to rejection is high. The data from this study demonstrate that BCL6 expression is more likely to be found in monomorphic PTLD than polymorphic PTLD. This finding, coupled with data previously reported by Hayashi, *et al.* on the association with monomorphic histology and poor response to RISM (6), highlights that early biopsy specimens from children in whom PTLD is considered may aid in management decisions for these patients. While we did not find a significant association between histologic subtype and response to RISM, only 15% of our patients with monomorphic disease responded to RISM, a percentage even lower than previously reported (6). Far fewer of the patients in the current cohort with polymorphic PTLD responded to RISM. The differences in our findings may reflect a difference in our definition of response to RISM.

The data from the recipients of heart transplants were particularly discouraging, as 4 of 8 died, and 3 of these were due to cardiac rejection or its treatment. Since only 1 of the heart transplant recipients responded to RISM long-term, this may be a population for whom RISM alone is used cautiously, particularly if they have BCL6 positive and monomorphic disease.

The major limitations of this study are the small size of the cohort and retrospective nature of the study. Thus, these findings will need to be confirmed in larger, prospective studies.

In conclusion, BCL6 expression is associated with monomorphic PTLD. These data suggest that the addition of BCL6 analysis to standard analysis of PTLD may help predict those patients that will require therapy beyond reduced immune suppression. Larger, prospective

studies may provide useful information about the significance of *BCL6* gene mutation and protein expression in children with PTLT.

Acknowledgments

This research was supported by the Colorado Pediatric General Clinical Research Center: Grant Number RR00069, General Clinical Research Centers Program, National Center for Research Resources, National Institutes of Health. The authors wish to thank Sara Garza-Williams and Lynne Meltesen for their help with immunohistochemistry and FISH, respectively.

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Figure 1. Morphologic features and Bcl6 expression in representative patients with post transplant lymphoproliferative disorders (PTLD)

A & B: Monomorphic PTLD (Patient 2), Burkitt lymphoma. **A:** Section shows a starry sky pattern and homogenous tumor cell population (H&E, $\times 400$). **B:** Strong positive nuclear staining in tumor cells (BCL6, $\times 400$).

C & D: Polymorphic PTLD (Patient 19). **C:** Section shows a heterogeneous lymphoid cell population varying from plasma cells and small lymphocytes to large transformed cells (H&E, $\times 400$). **D:** Negative staining in tumor cells (BCL6, $\times 400$).

Characteristics and response of individual patients

Table I

Pt	Gender	Age*	Type of Transplant	WHO Classification	Bcl-6 Expression (+/-) % of tumor cells	Response to RISM	Outcome (months from Dx)
1	m	1.7	liver	mPTLD, BL	(+) 40%	n	NED (96)
2	f	16.4	liver	mPTLD, BL	(+) 100%	n	NED (55)
3	m	0.9	liver	pPTLD	(-)	n	Died w/Dz (0)
4	f	3.2	liver	pPTLD	(+) 40%	n	Died w/Dz (1.5)
5	m	6.9	liver	PTLD, early lesion, Infectious mononucleosis-like type	(-)	y	NED (40)
6	f	0.8	liver	pPTLD	(-)	n	NED (81)
7	f	0.9	liver	mPTLD, BL	(+) 80%	n	NED (18)
8	m	0.5	liver	pPTLD	(-)	y	NED (50)
9a	m	1.1	liver	mPTLD, DLBCL	(-)	y [‡]	Relapse/NewDx (81)
9b				PTLD, classical HL	(+) 70%	n	NED (93)
10	f	8.3	liver	pPTLD	(-)	n	Relapse (3) Died w/Dz (30)
11	f	1.4	liver	pPTLD	na	n	Died w/Dz (0)
12	m	1.1	heart	pPTLD	(-)	n	Died w/Dz (0.5)
13	m	0.2	heart	malignant B cell lymphoma [‡]	(-)	n	Died w/Dz (0.5)
14	m	1.4	heart	mPTLD, high-grade lymphoma, not otherwise categorized	(+) 75%	n	Died w/Dz (5)
15	m	8.0	heart	mPTLD, DLBCL	(+) 90%	n	NED (31)
16	f	1.9	heart	mPTLD, high-grade lymphoma, not otherwise categorized	(+) 100%	n	Died w/NED (12)
17	f	3.1	HSCT	pPTLD	(-)	y	NED (17)
18	f	3.9	HSCT	pPTLD	na	n	NED (84)
19	m	5.5	HSCT	pPTLD	(-)	y	Died w/NED (58)
20	m	2.9	kidney	mPTLD, high grade lymphoma	(-)	n	NED (38)
21	m	15.7	heart	pPTLD	(-)	n	Relapse (12)/NED (19)
22	m	3.7	heart	pPTLD	(-)	y	NED (24)
23	f	7.8	kidney	mPTLD, DLBCL	(-)	n	NED (21)
24	m	15.2	kidney	mPTLD, DLBCL	(-)	n	Relapse (12)
25	m	2.5	heart	mPTLD, DLBCL	(+) 80%	y	Died w/NED (3)

* Age in years at time of transplantation

[†] specimen not available for re-classification

[‡] NED for 7 yrs after reduced immune suppression, then relapsed with histologically different lymphoma

OS=Overall survival

RFS=Relapse Free Survival

na – specimen not available for immunohistochemistry

mPTLD – monomorphic PTLD

pPTLD – polymorphic PTLD

BL – Burkitt's lymphoma

DLBCL – diffuse large B cell lymphoma

HL – Hodgkin's Lymphoma

Table II

Relationship between BCL-6 expression, histological type and response to RISM

	BCL6(+)	BCL6(-)	p-value
Histology			
Monomorphic	8	5	p=0.01
Polymorphic	1	10	
Response to RISM			
Yes	1	6	p=0.19
No	8	9	