The role of HLA mismatch, splenectomy and recipient Epstein-Barr virus seronegativity as risk factors in post-transplant lymphoproliferative disorder following allogeneic hematopoietic stem cell transplantation

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Background and Objectives. Post-transplant lymphoproliferative disorder (PTLD) following allogeneic hematopoietic stem cell transplantation (HSCT) is a fulminant disease with high mortality. The objective of this study was to determine risk factors in PTLD following HSCT in order to identify high-risk patients for surveillance, prophylaxis and treatment.

Design and Methods. Five hundred and fifty-three HSCT patients transplanted at Karolinska University Hospital in Huddinge between 1996 and 2004 were investigated retrospectively and 14 cases of PTLD were identified. Diseased patients were evaluated concerning transplantation procedure, PTLD diagnosis, treatment and outcome. Factors significant in univariate analysis were included in logistic regression multivariate analysis.

Results. The incidence of PTLD was 2.5% and the median onset of PTLD was 78 days post-transplantation. Only two PTLD patients survived. The most common therapy was anti-B-lymphocyte antibodies. Statistical analysis showed HLA mismatch ($p<0.001$), mismatch in Epstein-Barr virus (EBV) serology ($p<0.001$) and splenectomy ($p=0.006$) to be risk factors associated with PTLD. Indeed, among 387 patients with no risk factors only one developed PTLD (0.26%). Patients with one risk factor had a probability of developing PTLD of 8.2% and those with two risk factors, a probability of 35.7%.

Interpretation and Conclusions. We propose a strategy for dealing with PTLD. Patients without risk factors need not be monitored routinely. HSCT patients with one or more risk factors should be monitored weekly by polymerase chain reaction of EBV DNA, and for patients with two or more risk factors EBV-specific cytotoxic T-lymphocytes should be held in readiness before initiating the transplantation procedure.

Key words: EBV, post-transplant lymphoproliferative disorder, risk factors.

Haematologica 2006; 91:1059-1067

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Post-transplant lymphoproliferative disorder (PTLD) represents a wide spectrum of B-cell hyperproliferative states that include both benign conditions, e.g. infectious mononucleosis-like illness, and monoclonal malignancies, e.g. B-cell lymphomas that may be life-threatening. These lymphoid proliferations are almost invariably associated with T-cell dysfunction caused by the conditioning regimen for transplantation, and the presence of Epstein-Barr virus (EBV), thus allowing uncontrolled proliferation of EBV-infected B-lymphocytes.[1-4] EBV infects 90% of the world population. After a self-limiting primary infection, EBV remains latent in B-lymphocytes of healthy individuals.[5] PTLD occurs after both solid organ transplantation and allogeneic hematopoietic stem cell transplantation (HSCT) and the reported incidence ranges between 0.6% and 10%. However, some reports have suggested an incidence of PTLD of up to 24% in HSCT patients.[6-10] HSCT patients most commonly present with a fulminant and disseminated disease, which perhaps accounts for the increased mortality seen in this population. The mortality of HSCT patients in PTLD is approximately 80-90%.[6-10] The onset of the lymphoproliferative disorder usually occurs 70-90 days after the transplantation, although cases in which the onset has occurred several years later have been reported.[11,12]

Risk factors for the development of PTLD have been well studied in solid organ recipients, as reviewed by Cockfield *et al.* and Shroff and Rees.[13,14] In the late 1980s and 1990s, several studies reported that an HLA-mismatched donor and T-cell depletion of the graft are major risk factors in PTLD after HSCT. Mismatched grafts may be a source of chronic antigenic stimulation or delayed immune reconstitution, as in the case of T-cell depletion. Furthermore, immunosuppressive treatments, especially anti-T-cell agents such as OKT3 or anti-thymocyte globulin, have been associated with increased risk of developing PTLD.[6-10,15-17] These results have been re-investigated and new risk factors, including immun-

odeficiency and high age of the donor, have been added.[18,19] In solid organ transplant cases, a mismatch in cytomegalovirus (CMV) or EBV serostatus between recipient and donor has been found to increase the risk of PTLD.[20,21]

A definitive diagnosis of PTLD requires a tissue biopsy for morphological analysis, detection of EBV antigens by immunohistocytochemistry and EBV-encoded RNA (EBER) by *in situ* hybridization, and also determination of clonality of B-cell growth by analysis of immunoglobulin light chain type or rearrangement of the corresponding genes.[22-25] Prompt diagnosis is frequently necessary due to the rapid and often disseminated nature of PTLD in HSCT. Several attempts have been made to predict and survey the development of PTLD, e.g. serology for viral capsid antigens and T-cell counts. The most promising method appears to be quantification of EBV DNA in fluid or cellular samples. Greater than 500,000 viral genome copies per 100,000 peripheral blood lymphocytes is considered predictive of PTLD following solid organ transplants.[26-28]

Consensus is still lacking regarding the treatment of PTLD. The most common therapies involve reduction of immunosuppression as first-line treatment. Antiviral therapy, α-interferon treatment, chemotherapeutics, anti-B-cell (CD20) antibody therapies and *ex vivo* expanded cytotoxic EBV-specific T-lymphocytes (EBV-CTL) have been used in small studies.[7,10,19,29-33]

In this report, 14 cases of PTLD in 553 patients following HSCT were analyzed. Our aim was to discover risk factors for the development of PTLD, in order to determine whether a high-risk group of patients could be identified. Since it is difficult to predict when the EBV-driven lymphoproliferation will become truly malignant or behave in an aggressive, potentially fatal way, identification of high-risk patients is mandatory for well-targeted use of EBV-surveillance, possible prophylaxis and treatment of the appropriate patients.

Design and Methods

Patients

Between 1996 and 2004, 561 patients underwent HSCT at Karolinska University Hospital in Huddinge, Stockholm. Eight patients involved in a study of prophylactic treatment of PTLD using EBV-CTL were excluded.[33] A total of 553 patients were included in this survey. Most patients had hematologic malignancies, e.g. acute or chronic leukemia and lymphoma, but patients with solid tumors and inborn errors of metabolism were also included. One hundred and forty-nine patients (27%) were children (≤18 years) and 404 were adults. A review of the clinical research database, which contains systematically and prospectively collected data on all HSCT patients at Karolinska University Hospital in Huddinge, identified 14 cases of PTLD. All patients and/or their parents (in the case of young children) were advised of the procedures and attendant risks in accordance with institutional guidelines, and gave informed consent.

Transplantation procedure

Most patients had an HLA-A, -B and -DR identical unrelated donor or an HLA identical sibling, and a minority had mismatched related and unrelated donors. Before 1997, HLA class I typing was serological. Since then, low-resolution typing of class I with polymerase chain reaction (PCR) sequence-specific primers has been used. Starting in July 1992, PCR sequence-specific primers for HLA class II were employed.[34] All patients with unrelated donors have been re-typed retrospectively using high-resolution typing with PCR sequence-specific primers for both HLA class I and II antigens.[35]

The majority of conditioning regimens consisted of cyclophosphamide at 60 mg/kg on two consecutive days combined with 7.5–10 Gy of total body irradiation (TBI), with the lungs shielded to receive no more than 7–9 Gy (n=120),[36] fractionated (3 Gy for 4 days) TBI (n=124), busulphan at 4 mg/kg/day for four consecutive days (n=149), or total lymph node irradiation (n=1).[37] Reduced-intensity conditioning consisted of fludarabine at 30 mg/m^2/day for five consecutive days in combination with 2 Gy TBI (n=36), busulphan in a total dose of 8 mg/kg (n=68), cyclophosphamide in a total dose of 120 mg/kg (n=42), or treosulphan in a total dose of 10–14 mg/m^2/day for three consecutive days. A few patients were conditioned with only cyclophosphamide at a total dose of 200 mg/kg (n=7). All patients with unrelated or HLA-mismatched donors also received anti-T-cell prophylaxis consisting of anti-thymocyte globulin (Thymoglobuline®; Genzyme, Cambridge, MA, USA), anti T-lymphocyte globulin (ATG-Fresenius®; Fresenius AG, Bad Homburg, Germany), muromonab-CD3 (Othoclone OKT®3; Janssen-Cilag, Bridgewater, NJ, USA) or alemtuzumab (CamPath®; ILEX Pharmaceuticals, San Antonio, TX, USA) as a part of the conditioning regimen.[38]

The source of stem cells was bone marrow grafts in 234 patients, while the majority of the patients received stem cells mobilized to peripheral blood by granulocyte colony-stimulating factor (G-CSF).[39] Cord blood alone was the stem cell source in 13 patients, and in combination with a bone marrow graft in one patient. The median nucleated cell dose was 5.6×10^8/kg (range 0.03–80.0). Furthermore, G-CSF was given to 312 patients until absolute neutrophil counts reached 0.5×10^9/L.[40,41]

Most patients were given cyclosporine A combined with a short course of methotrexate as prophylaxis for graft-versus-host disease (GvHD). Cyclosporine A at an intravenous dose of 5-10 mg/kg/day in patients with matched unrelated donors and 1-3 mg/kg/day in patients with HLA-identical sibling donors was started one day before the transplantation.[42-44] During the first month, cyclosporine A levels in blood were kept at 200–300

Table 1. Characteristics of 553 patients following allogeneic hematopoietic stem cell transplantation.

	All patients	*PTLD patients*
N	*553*	*14*
Diagnosis		
Aplastic anemia	24 (4.3%)	1 (7.1%)
Acute leukemia	238 (43%)	5 (35.7%)
Chronic leukemia	105 (19%)	1 (7.1%)
Lymphoma	37 (6.7%)	3 (21.4%)
Myeloma	17 (3.0%)	0
Solid tumor	52 (9.3%)	0
Myelodysplastic syndrome	40 (7.2%)	3 (21.4%)
Inborn errors of metabolism	31 (5.6%)	1 (7.1%)
Patient sex (M/F)	312/241 (56.4/43.6%)	8/6 (57.1/42.9%)
Patient age (y), median (range)	37 (0-77)	12 (1-52)
Donor		
HLA-identical twin	5 (0.9%)	0
HLA-identical sibling	216 (39.1%)	0
HLA-identical related	5 (0.9%)	0
Mismatched related	7 (1.3%)	1 (7.1%)
Matched unrelated	257 (46.1%)	6 (42.9%)
Subtype mismatch unrelated	41 (7.4%)	4 (28.6%)
Mismatched unrelated	22 (4.0%)	3 (21.4%)
Nucleated cell dose ($\times 10^8$/kg), median (range)	5.6 (0.03-80.0)	6.0 (0.15-38.4)
Stem cell source		
Bone marrow	234 (42.3%)	6 (42.9%)
Peripheral blood stem cell	305 (55.1%)	7 (50%)
Cord blood	13 (2.6%)	1 (7.1%)
Cord blood and bone marrow	1 (0.2%)	0
Granulocyte colony-stimulating factor	312 (56.4%)	7 (50%)
GvHD prophylaxis		
None	5 (0.9%)	0
Monotherapy CsA	4 (0.7%)	0
CsA + MTX	439 (79.4%)	9 (64.3%)
T-cell depletion	12 (2.2%)	2 (14.3%)
Other	93 (16.8%)	3 (21.4%)
Conditioning		
Cy	7 (1.3%)	0
Cy + TBI 7.5-10 Gy	120 (21.7%)	1 (7.1%)
FTBI + Cy	124 (22.4%)	6 (42.9%)
Bu + Cy	149 (26.9%)	3 (21.4%)
RIC Flu + TBI 2 Gy	36 (6.5%)	1 (7.1%)
RIC Flu + Bu	68 (12.3%)	1 (7.1%)
RIC Flu + Cy	42 (7.6%)	2 (14.3%)
Others	7 (1.3%)	0
Anti-T-cell prophylaxis	379 (68.5%)	14 (100%)
Splenectomy	19 (3.4%)	3 (21%)
EBV Serology*		
Recipient +/-	471/70 (87.1/12.9%)	7/7 (50/50%)
Donor +/-	448/54 (89.2/10.8%)	13/1 (92.9/7.1%)
Donor+ to Recipient-	49 (8.9%)	7 (50%)
Donor- to Recipient+	36 (6.5%)	1 (7.1%)

*GVHD, graft-versus-host disease; CsA, cyclosporine A; Cy, cyclophosphamide; TBI, total body irradiation; FTBI, fractionated total body irradiation; Bu, busulphan; RIC, reduced intensity conditioning; Flu, fludarabine; EBV, Epstein-Barr virus. *Serological data for 12 recipients and 51 donors are missing for the overall series. The serological data are complete for the 14 patients who developed PTLD.*

ng/mL in patients with matched unrelated donors, and at approximately 100 ng/mL in patients with sibling donors. In recipients of grafts from unrelated donors the cyclosporine A dose was reduced after 3–6 months by 25% every month or every second month and discontinued after 6–12 months in the absence of GvHD. Using HLA-identical sibling donors, cyclosporine A was tapered after 2 months and discontinued after 3 months in the absence of GvHD.[44] Minor groups of patients did not receive any GvHD prophylaxis (syngeneic twins, n=5), or received monotherapy consisting of cyclosporine A. Other protocols included cyclosporine A combined with steroids (n=22) or mycofenolate mofentil (n=47).[42,45,46] Tacrolimus FK506 (Prograf®; Astellas Pharma Inc, Deerfield, IL, USA) in combination with mycofenolate mofentil (n=4), methotreaxate (n=9) or rapamycin (n=11) was also used in some patients (Table 1). *In vitro* T-cell depletion of the bone marrow graft was applied in 12 cases according to a previously described method.[47]

PTLD diagnosis

The diagnosis of PTLD was made according to the histological criteria reported for B-cell lymphoproliferative states following transplantation.[30] In two patients the diagnosis of PTLD was clinical (adenopathy, mass lesions, fever, unexplained pain etc), and corroborated with computed tomography scans. Twelve patients were tested by PCR of EBV DNA in peripheral blood lymphocytes or sera. The EBV serostatus of all donors and recipients was known before transplantation (Table 2).

Statistical analysis

Data were analyzed in July 2005. In the uni- and multivariate analyses, the logistic regression method was used. Factors that showed $p \leq 0.1$ in the univariate analysis were included in the backward elimination multivariate analysis. In the univariate analysis, the following factors were included: sex of recipient, age of recipient, lymphoma diagnosis, HLA mismatch, unrelated donor versus HLA-identical sibling donor, disease stage, second HSCT, nucleated cell dose, AB0 compatibility, sex of donor, age of donor, reduced intensity conditioning, anti-T-cell prophylaxis, acute GvHD, splenectomy, female donor to male recipient versus other combinations of genders, source of stem cells, CMV infection, CMV serostatus of donor and recipient, T-cell depletion, TBI-based conditioning, EBV serostatus of donor and recipient, and mismatch of EBV serostatus. The incidence of PTLD was estimated non-parametrically. Patients who died within one year after HSCT without developing PTLD were considered as competing event and patients surviving more than one year were censored. Analyses were performed using the *cmprsk* package (developed by Gray in June 2001), Splus version 6.2, and Statistica software.

Results

Incidence of PTLD and characteristics

PTLD occurred in 14 (2.5%) of the 553 patients following HSCT between 1996 and 2004. The median time of onset was 78 days (range 42–209) post-transplantation (Table 2). There were 12 histological or cytological diag-

Table 2. Characteristics of PTLD patients and their HSCT.

UPN	Diagnosis	Recipient Age*/Sex	Donor Age*/Sex	Match	Cell Source	Conditioning	EBV serology Donor	EBV serology Recipient	GvHD prophylaxis	GvHD	PTLD Diagnosis	PTLD DoD	Treatment	SE	Outcome
795	AML	4/M	32/M	MMRD[1]	BM	Bu+Cy	+	–	CsA+MTX	None	Clin + CT	50	Ritux		†, PTLD, d65
644	MDS	1/M	44/M	MUD	PBSC	Bu+Cy+Mel	+	–	CsA+MTX	None	Biopsy	58	Ritux	x	†, PTLD, d138
69	Fanconi	8/M	41/F	MUD	BM	Flu+Cy	+	–	Rapa+Fk	I	Biopsy	209	Ritux		A&W, 2y
824	MDS	13/M	28/M	MUD	BM	Cy+Flu	+	–	CsA+MTX	None	Biopsy	76	Ritux+DLI		†, Septic, d122
630	WAS	22/M	54/M	MUD	BM	FTBI+Cy	+	–	MP	I	Biopsy	47	EBV-CTL		†, PTLD, d63
726	CML	26/M	27/F	MUD	PBSC	FTBI+Cy	+	+	CsA+MMF	II	Biopsy	141	EBV-CTL		†, PTLD, d191
716	Hodgkin	40/M	44/M	MUD	PBSC	Flu+Cy	+	–	CsA+MTX	II	Biopsy	90	Ritux	x	†, PTLD, d101
868	ALL	10/F	24/F	MMUD[2]	PBSC	FTBI+VP16+Cy	+	–	CsA+MTX	I	Biopsy	68	Ritux+DLI		A&W, 6m
680	ALL	11/M	25/M	MMUD[2]	PBSC	FTBI+Cy	+	+	CsA+MTX	II	Biopsy	51	Ritux+DLI		†, Pneum, d123
808	ALL	37/F	29/F	MMUD[2]	PBSC	FTBI+Cy	+	+	CsA+MTX	I	Biopsy	90	Ritux		†, Pneum, d92
875	Hodgkin	37/F	54/F	MMUD[2]	PBSC	FTBI+Flu+Cy	+	+	CsA+MTX	I	Biopsy	42	Ritux	x	†, PTLD, d51
474	ALL	2/F	26/M	MMUD[1]	BM	FTBI+Cy	+	+	TcD+CsA+MTX	I	Biopsy	80	None		†, PTLD, d95
37	FHL	5/M	38/M	MMUD[1]	PBSC	TLI+VP16+Bu+Cy	+	+	TcD+CsA+MTX	None	Biopsy	138	EBV-CTL[3]		†, PTLD, d151
823	MDS	52/F	0/M	MMUD[1]	CB	FTBI+Flu+Cy	–	+	CsA+MMF	II	Clin + CT	154	Ritux		†, PTLD, d162

*UPN, unique patient number; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; Fanconi, Fanconi's anemia; WAS, Wiscott-Aldrich syndrome; Hodgkin, Hodgkin's disease; CML, chronic myeloid leukemia; ALL, acute lymphatic leukemia; FHL, familial hemophagocytic lymphohistiocytosis; *in years; M, male; F, female; Match, donor:recipient HLA match; MMRD, HLA mismatched related donor; MUD, matched unrelated donor; MMUD, HLA mismatched unrelated donor; [1]major HLA mismatch; [2]HLA subtype mismatch; BM, bone marrow; PBSC, peripheral blood stem cells; CB, cord blood; Bu, busulphan; Cy, cyclophosphamide; Mel, melphalan; Flu, fludarabine; FTBI, fractionated total body irradiation; TLI, total lymph node irradiation; VP16, etoposide; EBV, Epstien-Barr virus; GvHD, graft-versus-host disease; CsA, cyclosporine A; MTX, methotrexate; Rapa, rapamycin; Fk, tacrolimus; MP, methylprednisolone; MMF, mycofenolate mofentil; TcD, T-cell depletion; Clin + CT, clinical and computed tomography scan; DoD, day of diagnosis; Ritux, rituximab; DLI, donor lymphocyte infusion; EBV-CTL, EBV-specific cytotoxic T-lymphocytes; [3]dysfunctional cells; SE, splenectomized; †deceased; d, day; A&W, alive and well; Septic, septicemia; Pneum, pneumonia.*

noses and two clinical diagnoses, corroborated by computed tomography scans. Eight (57%) of the 14 patients who developed PTLD had received HLA-mismatched grafts, as opposed to 62 (12%) of the 539 non-PTLD patients ($p<0.0001$). All PTLD patients were given anti-T-cell prophylaxis. T-cell depletion was performed in two PTLD patients (14%) and in ten non-PTLD patients (1.9%) ($p=0.009$). Three (21%) of the patients in the PTLD group had undergone splenectomy prior to transplantation. The incidence of splenectomized patients in non-PTLD patients was 3.0% ($p=0.002$). EBV serostatus was investigated: seven (50%) of the recipients who developed PTLD were negative before the transplantation, as opposed to 63 (12%) of the non-PTLD patients ($p<0.001$) (data missing for 12). The frequency of mismatch in EBV serology, i.e. an EBV-positive donor and an EBV-negative recipient, was 50% in the PTLD group and 8.7% in the non-PTLD group of patients ($p<0.0001$).

Univariate analysis of risk factors in PTLD

In the univariate analysis, we found 11 factors ($p\leq0.1$) to be associated with PTLD following HSCT. These factors were young age of the recipient ($p=0.015$), lymphoma ($p=0.004$), HLA mismatch ($p<0.0001$), unrelated donor ($p=0.028$), anti-T-cell prophylaxis ($p<0.001$), splenectomy ($p=0.002$), CMV seropositivity of recipient ($p=0.026$), CMV seropositivity of donor ($p=0.07$), T-cell depletion ($p<0.009$), EBV seronegativity of recipient ($p<0.001$), and an EBV-seropositive donor for a seronegative recipient ($p<0.0001$) (Table 3).

Multivariate analysis of risk factors in PTLD

Backward elimination multivariate analysis was performed and identified HLA mismatch (OR 13.5, 95% CI 3.95–46.1, $p<0.001$), an EBV-seropositive donor for a seronegative recipient (OR 13.6, 95% CI 3.92–46.9, $p<0.001$) and splenectomy (OR 8.51, 95% CI 1.83–39.6, $p=0.006$) as significant risk factors for the development of PTLD after HSCT (Table 4).

The significant risk factors identified by the multivariate analysis were examined in greater detail. The cumulative incidence of PTLD in patients with an HLA-mismatched donor was 11.8%, as compared to 1.3% in those with an HLA-matched donor ($p<0.001$). EBV seronegative recipients who had had an EBV seropositive donor had a cumulative PTLD incidence of 14.3%, as compared to 1.6% for

Table 3. Results of the univariate analysis of risk factors associated with PTLD in 553 patients following HSCT.

Factor	*Competing events*	*Odds Ratio*	*95% CI*	*p*
Sex of recipient	male vs. female	0.97	0.33–8.83	0.96
Age of recipient	<18 y vs. >18 y	**3.76**	**1.28–11.1**	**0.015**
Lymphoma	lymphoma vs. no lymphoma	**4.02**	**1.07–15.1**	**0.04**
HLA mismatch (MM)	MM vs. matched	**10.4**	**3.50–31.2**	**< 0.0001**
Unrelated donor	unrelated vs. related donor	**9.82**	**1.27–76.0**	**0.028**
Disease stage	early vs. late	2.01	0.66–6.10	0.22
Second stem cell transplantation	first vs. second	2.32	0.66–8.19	0.19
Nucleated cell dose	continuous	1.01	0.96–1.07	0.60
ABO compatibility	compatible vs. non-compatible	1.06	0.56–2.00	0.86
Sex of donor	male vs. female	0.81	0.27–2.44	0.70
Age of donor	continuous	0.99	0.96–1.03	0.99
Reduced intensity conditioning (RIC)	RIC vs. no RIC	1.33	0.44–4.07	0.61
Anti-T-cell prophylaxis (ATG)	ATG vs. no ATG			**0.001**
Acute GvHD II–IV	0–I vs. II–IV	1.70	0.58–5.00	0.33
Splenectomy (SE)	SE vs. no SE	**8.91**	**2.26–35.2**	**0.002**
Female to Male (FtoM)	FtoM vs. no FtoM	1.02	0.44–2.33	0.96
PBSC	PBSC vs. BM and CB	1.22	0.67–2.24	0.50
CMV infection	CMV vs. No CMV	0.54	0.18–1.65	0.28
Recipient CMV seropositivity	CMV+ vs. CMV-	**0.30**	**0.10–0.87**	**0.026**
Donor CMV seropositivity	CMV+ vs. CMV-	**0.33**	**0.10–1.10**	**0.07**
T-cell depletion (TcD)	TcD vs. no TcD	**8.82**	**1.73–44.8**	**0.009**
TBI-based conditioning	TBI based vs. Non-TBI based	1.27	0.44–3.69	0.65
Recipient (R) EBV seropositivity	EBV+ vs. EBV-	**0.14**	**0.05–0.40**	**<0.001**
Donor (D) EBV seropositivity	EBV+ vs. EBV-	1.58	0.20–12.4	0.66
MM EBV serology	D EBV+ R EBV- vs. no EBV MM	**10.5**	**3.52–31.6**	**<0.0001**

GvHD, graft-versus-host disease; PBSC, peripheral blood stem cells; BM, bone marrow; CB, cord blood,CMV, cytomegalovirus; TBI, total body irradiation; EBV, Epstein-Barr virus.

all other combinations of donor/recipient EBV serostatus (p<0.001). Splenectomized and non-splenectomized patients had a cumulative incidence of PTLD of 15.8% and 2.1%, respectively (Figure 1).

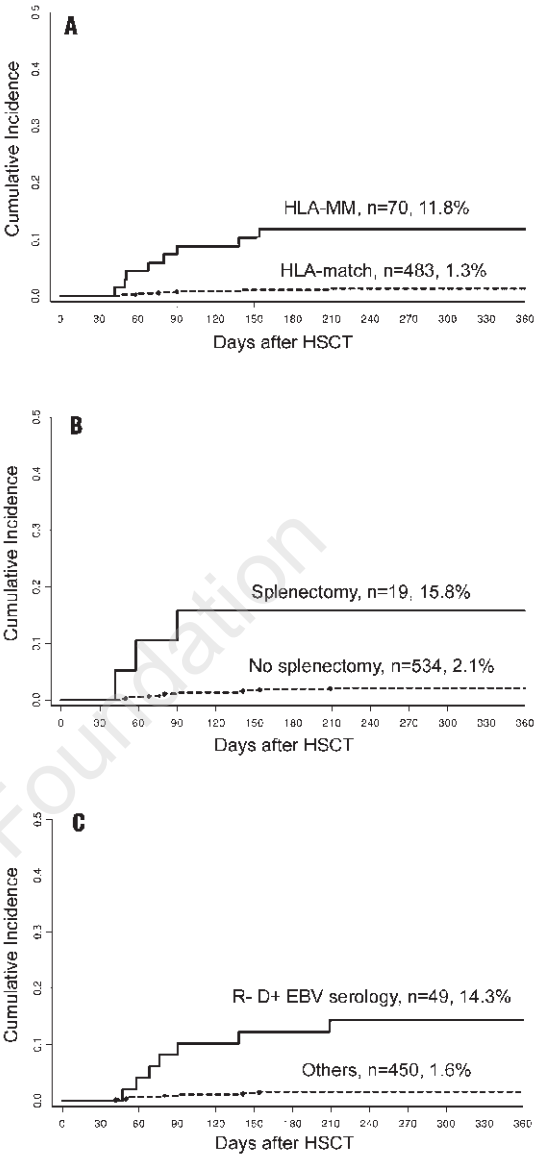

Figure 1. Time and cumulative incidence of PTLD in (**A**) patients receiving HLA-mismatched (HLA-mm) and HLA-matched grafts, (**B**) patients who had undergone splenectomy prior to transplantation and patients who had not undergone splenectomy, and (**C**) EBV-seronegative patients who received a graft from an EBV-positive donor (R^- D^+ EBV serology) versus all other patients.

Table 4. Results of the multivariate analysis of risk factors associated with PTLD in 553 patients following HSCT.

Factor	*Odds Ratio*	*95% CI*	*p*
HLA mismatch	13.5	3.95–46.1	<0.001
Mismatched EBV serology (R^-D^+)	13.6	3.92–46.9	<0.001
Splenectomy	8.51	1.83–39.6	0.006

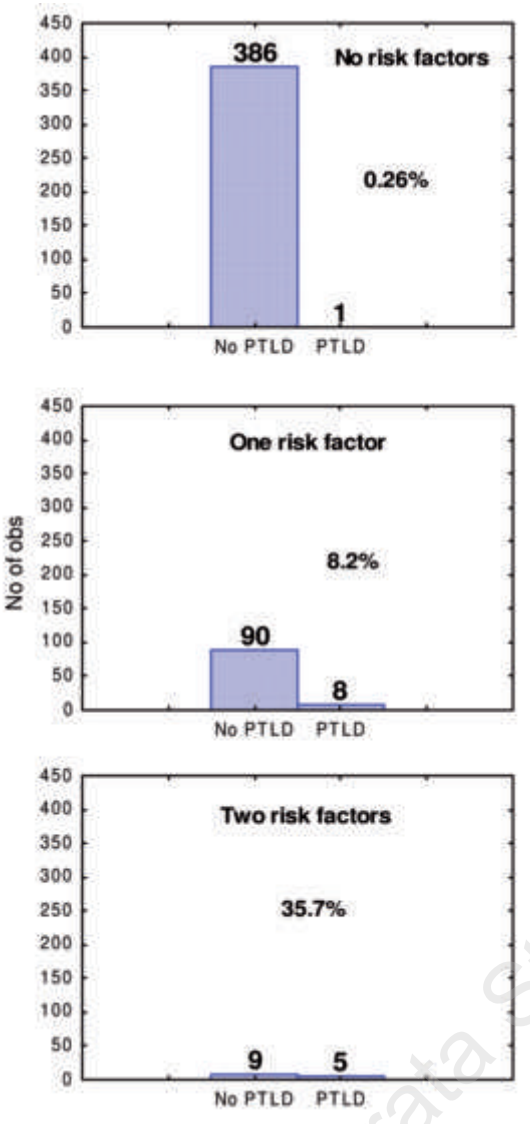

Figure 2. Among 387 patients with no risk factors (no HLA-mismatch, no splenectomy, no R- D+ EBV serology) only one developed PTLD (0.26%). In the presence of one or two of the above mentioned risk factors the incidences were 8.2% and 35.7%, respectively.

Among 387 patients with none of the risk factors identified in the multivariate analysis, one (0.26%) developed PTLD. Ninety-eight patients had one risk factor, and eight of them (8.2%) developed PTLD. Two risk factors were seen in 14 patients, and five of them (35.7%) developed PTLD (Figure 2).

Treatment and outcome of PTLD

On suspicion of PTLD, from the clinical syndrome or elevated EBV DNA, treatment with the anti-B-cell antibody rituximab (Mabthera®; Roche, Basel, Switzerland) was initiated in seven patients later verified as PTLD cases. Three patients received rituximab in combination with infusion of lymphocytes derived from the donor. Two patients were given EBV-CTL and one patient received dysfunctional EBV-CTL (which was confirmed later).[33] In one patient with rapid progression of PTLD, no treatment was started before death. Only two (14%) of 14 patients who developed PTLD survived. The two surviving patients were cured with rituximab alone or in combination with donor lymphocyte infusion. PTLD was the cause of death in nine of the 12 patients (Table 2). Two patients died from pneumonia, and one from enterococcal septicemia.

Discussion

In this report, 14 cases of PTLD following HSCT were identified (among 553 HSCT recipients) and investigated further. The majority of PTLD diagnoses were based on histology and only two cases were based on clinical features, corroborated by computed tomography scans. In this series, the overall incidence of PTLD was 2.5%. An incidence of up to 24% was reported by Shapiro *et al.* in a small group of recipients of T-cell-depleted and HLA-mismatched bone marrow grafts.[7] Gross *et al.* investigated 1,542 patients and reported an overall estimated incidence of PTLD following HSCT of 2.0%.[19] Furthermore, in a study from Helsinki, an incidence of 7.4% was found in recipients of HLA-matched and non-T-cell-depleted grafts.[18] The discrepancy in the reported incidences may reflect differences in the proportions of high-risk patients and/or in diagnostic criteria. The true incidence may be higher, since in other studies many cases have been revealed by autopsy.[19] The autopsy rate at our center is around 60%.[48] Despite the fact that most PTLD following HSCT may be disseminated and thus not diagnosed, we believe that few patients have been overlooked. The low incidence in this report may be explained by the low overall rate of T-cell-depletion (2.2%).

The median onset of disease in this series was 78 days post-transplantation, which is similar to the time of onset reported by others. The median time of onset in HSCT has been reported to be 70-90 days, as reviewed by Loren *et al.*[30] In a large American cohort, the investigators established that the average time of onset of PTLD in HSCT patients was 86 days post-transplantation.[19] In the Finnish study mentioned above, the PTLD patients lacked distinctive clinical features. All PTLD patients except one showed fever with a median onset at 72 days post-transplantation.[18]

The mortality rate in the PTLD group was 86%. Three of the 12 patients died after fulminant infections whereas nine deaths were caused directly by PTLD. It is difficult to establish the overall mortality due to PTLD, given the heterogeneity of presentations, underlying diseases and therapies. Zutter *et al.* reported a mortality rate of 93% in PTLD patients.[8] Other authors have reported comparable results: 92% (88% being directly caused by PTLD)[19] and approaching 90%.[31] The high mortality in our study may

be explained by a lack of experience in both EBV-PCR surveillance of HSCT patients and usage of rituximab-based treatment. Insufficient surveillance could lead to late interventions. Noteworthy, all PTLD patients received anti-T-cell prophylaxis, as also discussed below.

Our two surviving patients were treated with rituximab alone or in combination with donor lymphocyte infusion. Numerous different therapies against PTLD have been used over the years. Reduction of immunosuppression was initially described by Starzl *et al.*[29] and was confirmed as being useful by other investigators.[12] Antiviral agents such as ganciclovir and acyclovir have been used without striking results.[49,50] Gross *et al.* proposed treatment with α-interferon to restore the cytokine balance, i.e. to impair a milieu that favors proliferation of EBV-infected B-lymphocytes.[19] Anti-B-lymphocyte antibodies such as rituximab, and *ex vivo*-generated EBV-CTL have shown promising results in the treatment of PTLD.[31,32,51] The experience and outcomes with rituximab in both HSCT and solid organ transplantation have recently been reviewed. Rituximab was highly effective and favored as the second line of treatment after reducing the intensity of immunosuppression.[52] Moreover, early prophylactic usage of donor-derived EBV-CTL in high-risk patients with increasing amounts of EBV DNA in lymphocytes was successful.[33] HLA-matched EBV-CTL can be used as an alternative to donor-derived EBV-CTL.[53]

The univariate analysis identified nine risk factors associated with an increased risk of PTLD. These included HLA mismatch, anti-T-cell prophylaxis, T-cell depletion and unrelated donor transplants, which have all been described previously.[6-10,15-17,19] We also found recipient CMV seropositivity and mismatched EBV serology to be factors linked to an increased risk of PTLD. These have been described previously as risk factors in solid organ transplantation,[20,54] and our univariate data suggest that they may affect the incidence of PTLD after HSCT as well. The risk factors from the univariate analysis competed in the multivariate analysis. Three risk factors for development of PTLD remained significant: HLA mismatch, graft from an EBV-seropositive donor to a seronegative recipient, and splenectomy. Some of the significant risk factors in the univariate analysis may be important, but they were not significant in the multivariate analysis because of the small number of observations. Data from large series collected in multiple centers may help to elucidate this.

HLA mismatch may have a role in the pathogenesis of PTLD because immune reconstitution is delayed following a mismatched graft and T-lymphocyte immunity is of major importance in the control of EBV. Furthermore, it has been hypothesized that mismatched grafts may lead to chronic antigenic stimulation.[7,19] As indicated above, HLA mismatch is regarded as a well-known risk factor in PTLD. According to our results, EBV-seronegative patients receiving grafts from seropositive donors are at particular risk. In almost all cases of PTLD following HSCT the aberrant lymphocyte proliferation is donor-derived, as the recipient's lymphoid system is eradicated by the conditioning regimen. Even in cases in which the donor was EBV-seronegative, the PTLD was shown to be of donor origin.[7,8,55] Thus, mismatched EBV serology being a risk factor in PTLD must be taken into consideration throughout the HSCT procedure.

We also identified splenectomy as a risk factor in our analysis. Splenectomy has not been reported as a risk factor in PTLD previously. These data need to be approached with caution, since only 19 patients underwent splenectomy in our study and three of them developed PTLD. However, an increased risk of PTLD after splenectomy could be due to an impaired ability to delete deficient B-lymphocytes. Naïve EBV-infected B-lymphocytes may escape selection in germinal centers, e.g. through downregulation of surface expression.[56-58]

All patients in our study who developed PTLD received anti-T-cell prophylaxis. Thus, we were unable to evaluate anti-T-cell prophylaxis in the multivariate analysis. The incidence of PTLD in patients receiving anti-T-cell prophylaxis was 3.7%. Anti-T-cell prophylaxis has been strongly associated with an increased risk of PTLD because of the slow recovery of T-lymphocyte numbers.[6-10,15,16] As in our series, in the study by Juvonen *et al.*, all patients who developed PTLD had also received anti-T-cell prophylaxis.[18]

This study confirms that the risk of developing PTLD, an often fatal complication of HSCT, may be predicted by risk factors. Our results implicated HLA mismatch, mismatched EBV serology and splenectomy as significant risk factors. Further investigations showed a low risk (0.26%) of development of PTLD if no factor was present. If one or two factors were present, the incidence of PTLD increased to 8.2% and 35.7%, respectively. No consensus regarding treatment and monitoring of PTLD has been proposed so far, and prophylactic use of donor lymphocyte infusions or EBV-CTL is time-consuming and costly.[30] Thus, we propose the following strategy. For patients with no risk factor, routine surveillance is not necessary. Patients with one risk factor present should be monitored with PCR of EBV DNA every week or every other week. If the EBV DNA level increases by several logs, rituximab should be given and EBV-CTL should be prepared. Prophylactic administration of rituximab during HSCT could be one way of preventing PTLD.[51] Patients with two or more risk factors should be monitored every week with PCR and EBV-CTL should be held in readiness before HSCT. This suggestion is based on the risk factors reported by us, but it is possible that other risk factors such as anti-T-cell prophylaxis and T-cell depletion should be included. Surveillance, as proposed, will lead to earlier intervention and, it is to be hoped, reduce PTLD-related deaths.

MS designed the study in co-operation with the other authors. CL collected clinical data in co-operation with LB and MS. MR was responsible for the statistical analyses. The results and manuscript draft were completed by MS. KLB, OR and MR were in charge of the study design, the analysis of results and the preparation of the manuscript. All co-authors actively participated in the preparation of the manuscript. The authors declare that they have no potential conflict of interests. This study was supported by grants from the Swedish Cancer Society (0070-B05-19XAC and 4562-B05-05XBB), the Children's Cancer Foundation (03/039, 03/007, 05/077), the Swedish Research Council (K2006-32X-05971-26-1, and K2006-32X-14716-04-1), the Tobias Foundation, the Swedish Society of Medicine, the Sven and Ebba-Christina Hagbergs Foundation, the Cancer Society of Stockholm, and Karolinska Institutet. Manuscript received February 10, 2006. Accepted May 30, 2006.

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