ORIGINAL ARTICLE

Post-transplant lymphoproliferative disorder following kidney transplantation: a population-based cohort study

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Key words
Epstein–Barr virus, graft survival, kidney transplantation, mortality, post-transplant lymphoproliferative disorder

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Conflicts of interest
The authors declare no conflict of interests.

Received: 12 May 2015
Revision requested: 3 July 2015
Accepted: 30 December 2015
Published online: 18 February 2016

SUMMARY

Post-transplant lymphoproliferative disorder (PTLD) incidence is difficult to determine, mainly because both early and other lesions may go unrecognized and unregistered. Few studies have included systematic pathology review to maximize case identification and decide more accurately PTLD frequency after long-term post-transplantation follow-up. A retrospective population-based cohort study including all kidney transplant recipients at two Danish centres (1990–2011; population covered 3.1 million; 2175 transplantations in 1906 patients). Pathology reports were reviewed for all patient biopsies to identify possible PTLDs. Candidate PTLDs underwent histopathological review and classification. Seventy PTLD cases were identified in 2175 transplantations (3.2%). The incidence rate (IR) after first transplantation was 5.4 cases per 1000 patient-years (95% CI: 4.0–7.3). Most PTLDs were monomorphic (58.5%), or early lesions (21.5%). Excluding early lesions and patients <18 years, IR was 3.7 (95% CI: 2.9–5.5). Ten patients with PTLD were retransplanted, 2 developing further PTLDs. Post-transplant patient survival was inferior in patients with PTLD, while death-censored graft survival was not. Using registry data together with extensive pathological review and long follow-up, a rather high incidence of PTLD was found.

Transplant International 2016; 29: 483–493

doi:10.1111/tri.12744

Introduction

Post-transplant lymphoproliferative disorder (PTLD) is an important complication of the changed immunological environment after transplantation. PTLDs are uncontrolled plasmacytic or lymphoid proliferations, comprising a morphologically heterogeneic group of early (often self-limited), polymorphic and monomorphic (lymphoma-like) lesions [1]. Risk factors for developing PTLD include degree and type of immunosuppression, recipient age, dialysis duration and, possibly, a high number of HLA mismatches [2–4]. The most important factor is primary post-transplantation Epstein–Barr virus (EBV) infection [5–7].

EBV genes and gene products can often be detected in PTLD tissues, although the reported incidence of EBV association varies considerably (40–90%) between studies [2,8,9]. These virus-driven proliferations appear to develop as a result of impaired T-cell immunity to new or reactivated EBV infections in immunosuppressed transplanted patients [4,8,10].

Most reports suggest that PTLDs are relatively rare. The risk following kidney transplantation (often poorly defined) varies between 1 and 3% [1,5,11,12], although it has been reported up to 7% [13,14]. PTLD development is associated with decreased survival of both patients and grafts [11], 5-year patient survival after diagnosis ranging between 37 and 65% [15–18].

PTLD studies have focused mainly on data either from overt cases, not always including early lesions [12,19,20], or from particular age groups [2,11,21–23]. Studies may show selection in favour of overtly malignant cases [16] or may only include cases registered in cancer databases. To our knowledge, no in-depth report of PTLD following kidney transplantation, including systematic review of all recipients at risk, has been published. We conducted a retrospective population-based cohort study with meticulous review of all renal transplant recipients over two decades, to clarify incidence, pretherapeutic clinical features, clinical course and outcome of PTLD in the 3.1 million inhabitants of western Denmark. This allowed us to include essentially all identifiable cases of PTLD, including early lesions and less obvious cases, allowing a more accurate evaluation of incidence and outcome.

Patients and methods

This population-based cohort study included all kidney recipients transplanted at our two centres in western Denmark between 1 January 1990 and 31 December 2011. All Danish kidney transplants are recorded in the Danish Nephrology Registry [24]. Using this, we identified a total of 2175 transplantations in 1906 patients within the study period, including 229 and 20 patients with, respectively, two or three transplantations.

The Danish national health system provides publicly funded medical care for all residents. Each resident has a unique personal identification number (the CPR number) that allows linkage of Danish clinical databases and registries, facilitating complete long-term follow-up of patients in epidemiological studies. Pathological analyses are recorded in the Danish National Pathology Registry [25]. This registry has a high degree of completeness and includes records (with detailed histopathological descriptions) of essentially all diagnostic pathology studies performed throughout Denmark, updated in real time. Registry information was complete for the participating pathology departments during the study period [25]. Using CPR numbers, we searched the Pathology Registry for relevant histopathological biopsies (including autopsy specimens) obtained from the 1906 cohort patients (Table S1). Within the study period, we identified 4107 histopathological investigations in cohort patients. These were reviewed, and specimens fulfilling the diagnostic criteria for PTLD were identified [1]. Cases not initially diagnosed as PTLD, but in which review of the original histopathology report aroused suspicion of possible lymphoproliferation, underwent formal microscopy review. In these cases, archived paraffin blocks (when available) were obtained from the primary pathology department, and new stains were performed to clarify the diagnosis.

Cases of PTLD were revised and classified according to the 2008 WHO classification [1]. PTLD tissues were classified as EBV negative or positive according to the results of viral histopathological analysis performed either at original diagnosis or during our review. EBV-positive cases showed an appropriate positive stain for EBV latent membrane protein (LMP-1) (EBV-LMP clone CS.1-4, Dako, Glostrup, Denmark), EBV nuclear antigen-2 (EBNA-2 clone PE2, Abcam, Cambridge, UK) or EBV-encoded small RNAs (EBERs; INFORM EBER Probe, Ventana Medical Systems, Tucson, AZ, USA).

PTLD patient data were extracted by retrospective chart analysis. For all patients diagnosed with PTLD, a search was made in the Scandiatransplant Database and the Danish Lymphoma Group Registry. We defined EBV-negative serostatus as a negative measurement at, or not more than 3 months before, transplantation. PTLD was defined as a lymphoproliferative disorder developing at least 1 month after transplantation and

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not later than 2 years after loss of graft function. Cases were only included after graft loss, if the patients still received immunosuppressive treatment at the time of diagnosis. Date of PTLD diagnosis was defined as the date on which the pathologist received the PTLD tissue. Time to tumour onset was defined as the time between the most recent transplantation and PTLD date. In patients with several PTLD lesions, only the first PTLD after each transplantation was included.

Early-onset PTLD was defined as PTLD developing within the first year after transplantation; all other cases were defined as late-onset PTLD. A flow chart describing the work process is presented in Fig. S1.

Statistical analysis

Patient-years (Pt-yrs) of follow-up for risk analysis were calculated from 1 month after transplantation to graft loss, death, PTLD diagnosis, emigration or 31 December 2011, whichever came first. Incidence rate (IR) was calculated for each transplantation number. One hundred and eighteen patients (all without PTLD) were excluded from the incidence analysis, as their follow-up was <1 month. The total follow-up time for patients with their first transplantation between 1990 and 2011 (n = 1392) was 77 961 years (average 6.4 years). Comparison of patient characteristics for transplantations with and without subsequent PTLD, and early- and late-onset PTLD patients was made using chi-square testing and Fischer′s exact test for dichotomous data, and Student's t-test for continuous variables. Cumulative incidence was analysed using death and graft loss as competing risk [26].

Progression-free survival (PFS) was calculated from the date of PTLD diagnosis to date of death, relapse/progression, loss to follow-up or end of study, whichever came first. Overall survival (OS) was computed from date of PTLD diagnosis to date of death, loss to follow-up or end of study, whichever came first. PFS and OS curves were estimated by Kaplan–Meier and tested by log rank. Both analyses were censored for loss to follow-up or end of study.

Post-transplant patient and graft survival were compared between PTLD and non-PTLD patients with their first transplantation between 1990 and 2011 by landmarking analysis after 1, 2, 3 and 5 years, stratified for transplantation centre and age at transplantation [27]. Graft survival was also stratified for donor's age and the degree of HLA mismatch. Pt-yrs for post-transplant patient survival were calculated from the date of transplantation to end of study or death. Pt-yrs for post-transplant graft survival were calculated from the date of transplantation to end of study or graft loss including death from any cause with functioning graft (non-death-censored). Pt-yrs for death-censored graft survival were calculated from the date of transplantation to end of study or graft loss censoring for death with functioning graft.

Univariate associations between clinical parameters of interest and the risk of PTLD were investigated using Cox proportional hazards model for patients with their first transplantation between 1990 and 2011 (n = 1392). All variables with a significant P-value in the univariate analysis were entered into a multivariate Cox proportional hazards model stratified for transplantation centre. The proportional hazards assumption was tested by introducing time-varying hazard ratios (more specifically separate hazard ratios up to 2 years). No evidence of violation of the proportional hazards assumption was found.

Data management and analysis were performed using STATA IC 12.1 (2012), StataCorp, College Station, TX, USA. Graphical presentations were performed using SIGMAPLOT 12.5 (Systat Software, San Jose, CA, USA). All P-values were 2-sided and considered statistically significant if < 0.05.

Results

PTLD patient demographics

In the study period 1990–2011, 2175 kidney transplantations were performed, associated with 70 cases of PTLD (3.2%), including 48 cases in 1363 men (3.5%) and 22 cases in 812 women (2.7%). Mean age at transplantation was 34.7 years (range 1.3–68.1 years). A summary of baseline demographics for all transplant recipients is given in Table 1.

After biopsy reviews, 2 additional PTLDs were found, one patient with a second PTLD after re-transplantation and one patient with an unregistered case of PTLD (see Fig. S1).

Eight patients developed PTLD after loss of graft function. Three of them were diagnosed with PTLD more than a week after graft loss (6.5, 22.5 and 23 months, respectively).

PTLD incidence

The PTLD IR following first transplantation was 5.4 cases per 1000 Pt-yrs (95% confidence interval [CI]: 4.0–7.3). The incidence changed according to time after transplantation, showing a bimodal pattern with highest IRs in the first year and more than 10 years after transplantation (Fig. 1a). IR for patients aged 18 or older at transplantation was 4.5 per 1000 Pt-yrs (95% CI: 3.2–

Table 1. Baseline demographics for all kidney transplantations.

	Transplantations with subsequent PTLD (n = 70)	Transplantations without subsequent PTLD (n = 2105)
Gender		
Female	22 (31.4)	790 (37.5)
Male	48 (68.6)	1315 (62.5)
Missing data	0 (0)	0 (0)
Age at transplantation, years		
0–17	12 (17.1)	129 (6.1)
18–35	27 (38.6)	551 (26.2)
36–53	20 (28.6)	879 (41.8)
>53	11 (15.7)	546 (25.9)
Missing data	0 (0)	0 (0)
Transplantation year		
1990–1994	24 (34.3)	443 (21.1)
1995–1999	15 (21.4)	406 (19.3)
2000–2004	16 (22.9)	462 (22.0)
2005–2011	15 (21.4)	794 (37.7)
Missing data	0 (0)	0 (0)
Donor status		
Deceased	30 (42.9)	1160 (55.1)
Living	19 (27.1)	525 (24.9)
Missing data	21 (30.0)	420 (20.0)
Degree of HLA mismatch: A, B, DR		
0	6 (8.6)	123 (5.8)
1–3	32 (45.7)	1063 (50.5)
4–6	11 (15.7)	499 (23.7)
Missing data	21 (30.0)	420 (20.0)
Epstein–Barr virus serostatus before tx, patient		
Recipient negative	10 (14.3)	54 (2.6)
Recipient positive	5 (7.1)	981 (46.6)
Missing data	55 (78.6)	1070 (50.8)
Epstein–Barr virus serostatus before tx, donor		
Donor negative	0 (0.0)	37 (1.8)
Donor positive	9 (12.9)	561 (26.7)
Missing data	61 (87.1)	1507 (71.6)
Epstein–Barr virus serostatus before tx, recipient and donor		
Recipient positive, donor positive	1 (1.4)	462 (21.9)
Recipient positive, donor negative	0 (0.0)	24 (1.1)
Recipient negative, donor positive	5 (7.1)	12 (0.6)
Recipient negative, donor negative	0 (0.0)	2 (0.1)
Missing data	64 (91.4)	1605 (76.2)

6.3), and for patients <18 years, it was 15.7 per 1000 Pt-yrs (95% CI 8.5–29.2). In subanalysis, the incidence of PTLD excluding early lesions was 4.2 per 1000 Pt-yrs (95% CI 3.0–6.0), and when excluding both patients <18 years and early lesions, IR was 3.7 (95% CI: 2.9–5.5). The IR of early-onset PTLD did not change over the 22-year period studied (P = 0.812). Cumulative incidence is shown in Fig. 1b. The cumulative incidence was 1.4% (95% CI: 0.8–2.2) and 2.2% (95% CI: 1.4–3.3) after 5 and 10 years, respectively.

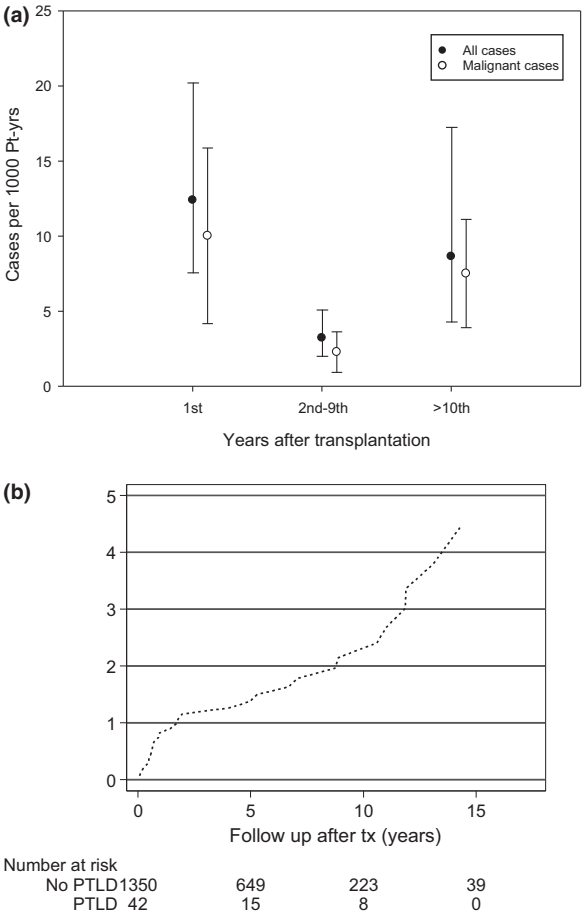

Figure 1 PTLD incidence rates. (a) Incidence according to time after transplantation, all cases and malignant cases (monomorf, polymorph and Hodgkin's subtype). (b) Cumulative incidence rate.

Time to PTLD

The median age at PTLD diagnosis was 39.5 years (range: 2.1–78 years). Nineteen cases (27.1%) were early-onset PTLD. Fifty-one cases (72.9%) were late-onset PTLDs, and late-onset PTLD occurred with a high incidence from 10 years after transplantation (Fig. 1a).

Early-onset PTLDs were more often associated with location in the graft (P = 0.001), use of tacrolimus (P = 0.032) and exclusively extranodal disease (P = 0.022). On the contrary, late-onset PTLDs were more frequently associated with nodal disease (P = 0.014) and use of cyclosporine (P = 0.015). There were no differences in PFS and OS comparing the early- and late-onset group (P = 0.902 and P = 0.559, respectively).

Morphological classification and localization of PTLD

Most cases were monomorphic PTLDs (38 of 65; 58.5%), the majority being diffuse large B-cell lymphomas (DLBCL, $n = 33$). There were no cases of T-cell lymphoma. Fourteen PTLDs (22%) were early lesions [1], and all early lesions showed involvement of lymph nodes. Nine of 14 patients with early lesions were under the age of 36 at the time of transplantation (6 patients <18 years). Lymph nodes were the most common localization (65.7%) for all patients, followed by the gastrointestinal tract (15.6%). For additional PTLD characteristics, see Table 2.

Epstein–Barr virus status of PTLD tissue

Sufficient PTLD tissue was available for analysis of EBV status in 63 cases. Forty-seven PTLDs (74.6%) were positive for EBV (in the lymphoproliferation). Fifteen of 17 early-onset PTLDs (88.2%) were EBV positive, compared with 32 of 46 late-onset PTLDs (69.6%). EBV was positive in all PTLDs of classical Hodgkin lymphoma subtype.

PTLD treatment

Information on PTLD treatment was available for 63 patients. Reduction of immunosuppressive therapy was included as part of first-line therapy in 57 of 63 patients (90%). Sixteen patients were treated with reduction in immunosuppression alone, including eight patients diagnosed with early lesions and four patients with monomorphic PTLD. Two patients died from progressive disease after being treated with reduced immunosuppression alone. One had a progressive DLBCL; the other was diagnosed with incurable DLBCL of the CNS, 51 months after initial diagnosis of a nodal early lesion. One patient diagnosed in December 2011 had disease evaluation after the end of the study period. Fourteen patients had a complete remission (CR). Treatment with reduced immunosuppression alone was not associated with poorer PFS or OS ($P = 0.143$ and $P = 0.210$) compared with other treatment modalities.

Rituximab treatment was administered in 29 of 65 cases (45%), with a median number of 4 treatment cycles (range 1–21). Other treatment forms are described in Table 2.

Seven patients (10%) received no treatment for their PTLD. Three of these patients were diagnosed postmortem, one declined treatment, one was terminal, one commenced treatment after the end of the study, and

Table 2. Patients with PTLD.

	Missing		PTLD cases ($n = 70$)	
	No.	%	No.	%
Immunosuppression*				
Induction therapy				
Antithymocyte globulin	3	4.3	36	53.7
Prednisolone	13	18.6	24	42.1
Anti-IL2-receptor-antibody	1	1.4	24	34.8
Maintenance therapy				
Prednisolone	9	12.6	39	63.9
Mycophenolate mofetil	6	8.6	48	75.0
Azathioprine	11	15.7	17	28.8
Cyclosporine A	5	7.1	55	84.6
Tacrolimus	10	14.3	24	40.0
Sirolimus	10	14.3	4	6.7
Rejection therapy				
Methylprednisolone	9	12.6	17	27.9
Antithymocyte globulin (ATG)	9	12.6	5	8.2
Anti-CD3 monoclonal antibody (OKT3)	8	11.4	6	9.7
Age at PTLD diagnosis, years	0	0		
0–17			7	10.0
18–35			24	34.3
36–53			24	34.3
>53			15	21.4
PTLD year	0	0		
1990–1994			7	10.0
1995–1999			10	14.3
2000–2004			14	20.0
2005–2011			39	55.7
PTLD localization				
Kidney graft	4	5.7	5	7.6
Lymph node	3	4.3	44	65.7
Gastrointestinal tract	6	8.6	10	15.6
Bone marrow	6	8.6	5	7.8
CNS	6	8.6	3	4.7
Tonsil (palatine or lingual)	6	8.6	10	15.6
Lung	6	8.6	5	7.8
Liver or pancreas	6	8.6	5	7.8
Spleen	6	8.6	9	14.1
Pharynx or salivary gland	6	8.6	5	7.8
Other	6	8.6	14	21.9
Disease status	6	8.6		
Single site			40	62.5
Only nodal			24	37.5
Only extranodal			23	35.9
Both nodal and extranodal			17	26.6
B symptoms†	10	14.3		
Present			20	33.3
WHO 2008 classification	5	7.1		
Early lesions			14	21.5
Polymorphic PTLD			10	15.4
Monomorphic PTLD			38	58.5
DLCBL			33	50.8
Burkitt lymphoma			3	4.6
Other‡			2	3.1
Classical Hodgkin lymphoma-type PTLD			3	4.6
Epstein–Barr virus status of the PTLD tissue	7	10.0		
Positive			47	74.6
Negative			16	25.4

Table 2. Continued.

	Missing		PTLD cases (n = 70)	
	No.	%	No.	%
Treatment§				
RI	7	10.0	57	90.5
Rituximab	5	7.1	29	44.6
Radiotherapy	5	7.1	8	12.3
Chemotherapy	6	8.6	20	31.3
CHOP			10	15.6
AVBD			2	3.1
CNS triple			2	3.1
Other			6	9.4
Surgery	4	5.7	11	16.7
Graftectomy			3	4.5
No treatment	2	2.9	7	10.3
Treatment grouped§	5	7.1		
RI alone			16	24.6
RI+Rituximab			15	23.1
RI+chemotherapy			3	4.6
RI+Rituximab+chemotherapy			9	13.8
RI+surgery			5	7.7
Other combinations			10	15.4
No treatment			7	10.8
Response	7	10.0		
Complete remission			52	82.5
Partial remission			3	4.8
Progressive disease			4	6.4
Other			4	6.4
Renal function at the end of follow-up¶	0	0		
Functioning graft**			45	64.3
Loss of graft function			25	35.7

PTLD, Post-transplant lymphoproliferative disorder; DLBCL, diffuse large B-cell lymphoma; RI, reduced immunosuppression; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; AVBD, adriamycin, bleomycin, vinblastine, dacarbazine; CNS triple, methotrexate, cytarabine, prednisolone.

*The mentioned immunosuppression is ever use; the same patient might be included in both, for example, cyclosporine and tacrolimus.

†Defined as weight loss >10% of habitual weight within 6 months, febrilia or night sweats.

‡Other monomorphic PTLDs were 1 case of unspecified monomorphic PTLD and 1 case of plasma cell myeloma.

§The treatment described includes both first-line and relapse treatment.

¶defined as end of study or death, whichever came first.

**including death with functioning graft.

one was not identified as having a PTLD (an early lesion) until inclusion in this study. Therefore, the latter was not treated for the lymphoproliferation, as this was not recognized at the time of biopsy. Thus, this patient was able to clear the PTLD without any intervention.

Patients with multiple PTLD episodes

Two patients had two pathologically distinct PTLDs in the course of two separate transplantations. One patient had a polymorphic PTLD arising in the first graft 2 months after transplantation and a nodal early lesion PTLD occurring 9 years after the second transplantation. The other patient developed a DLBCL in the rhinopharynx and adjacent tonsils, 3 months after the first transplantation and an EBV-positive mucocutaneous ulcer PTLD in the mouth 27 months after the second transplantation.

A third patient experienced an early nodal lesion 159 months after transplantation with relapse of early lesions in the palatine tonsil and in the palate after intervals of 37 and 12 months, respectively. Two months later, the patient was diagnosed with CNS-DLBCL, dying within a few days.

A fourth patient developed a nodal DLBCL 70 months after transplantation. Twenty-two months after the initial PTLD diagnosis, the patient developed a second PTLD, compromising an early lesion in a lymph node.

Retransplantation

Ten patients were retransplanted after the PTLD diagnosis and treatment. The median time from diagnosis to retransplantation was 5.4 years (range: 2.9–8.7 years). As described, two patients had a second PTLD after retransplantation. One of the ten patients was retransplanted twice, after 8.7 and 13.1 years, neither of which was complicated by PTLD.

Response

The overall treatment response rate was 87% (55 of 63). CR was achieved in 52 patients (83%) and partial remission in three (5%). Response rate was highest in early lesions (100%) followed by polymorphic cases (90%). A total of 27 patients died during the follow-up period. Twelve died of PTLD within a range from 0 to 1576 days from diagnosis to death. One patient died of an unrelated cancer, seven died of infection, and seven died for other reasons.

Ten patients (14%) had a relapse. Time from PTLD diagnosis to relapse ranged from 3 to 53.7 months. Two patients had two separate relapse episodes: one had relapses after 2.5 and 5.5 years and the other 3.1 and 4.1 years after the initial diagnosis. PFS at 1, 5 and 10 years was 79%, 59% and 52%, respectively. OS at 1, 5 and 10 years was 81%, 64% and 57% (Fig. 2).

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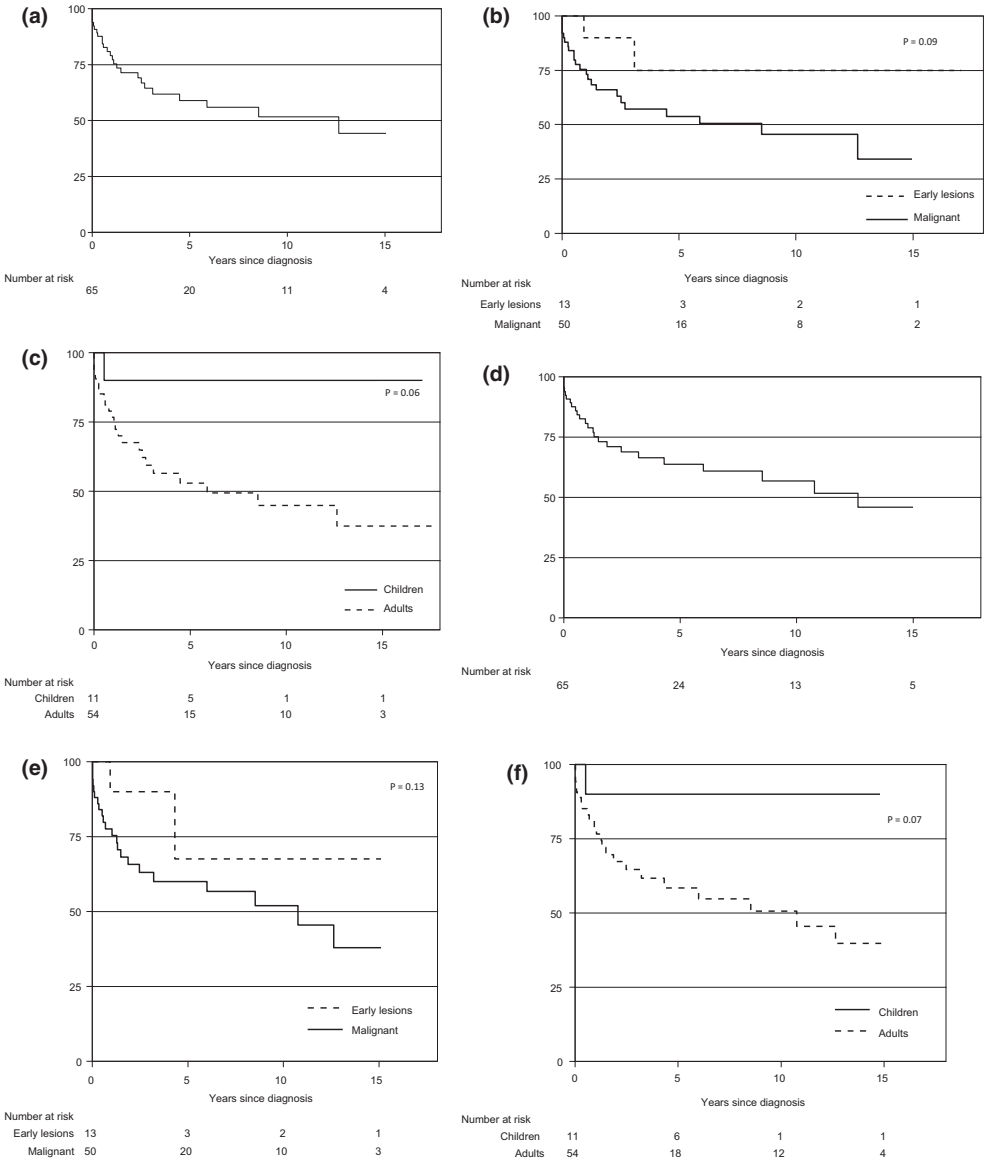

Figure 2 Progression-free and overall survival after PTLD diagnosis. (a) Kaplan–Meier curve of progression-free survival (PFS) after post-transplant lymphoproliferative disorder. (b) Progression-free survival stratified for type of lesion, early lesion versus malignant cases (monomorf, polymorph and Hodgkin's subtype). (c) Progression-free survival stratified for age at transplantation, children (<18) versus adults (≥18). (d) Kaplan–Meier curve of overall survival (OS) after post-transplant lymphoproliferative disorder. (e) Overall survival stratified for type of lesion, early lesion versus malignant cases (monomorf, polymorph and Hodgkin's subtype). (f) Overall survival stratified for age at transplantation, children (<18) versus adults (≥18).

Mortality and graft loss

Patients with PTLD occurring before 1, 2, 3 or 5 years after transplantations had a higher risk of death compared to patients with no occurrence of PTLD before these time points, the hazard ratios (HR) being 4.87 (95% CI: 1.95–12.16), 4.41 (95% CI: 1.91–10.16), 5.44 (95% CI: 2.63–11.29) and 5.82 (95% CI: 2.98–11.37).

The risk of graft loss with death-censored graft survival was not significantly higher in patients with PTLD, but tended to be better more than 1 year after transplantation, with HR after 1, 2, 3 and 5 years being 1.05 (95% CI: 0.25–4.47), 0.84 (95% CI: 0.2–3.54), 0.65 (95% CI: 0.16–2.73) and 0.62 (95% CI: 0.15–2.59). Graft loss, including death with functioning graft, was higher in patients with PTLD 5 years after transplantation, with HR after 1, 2, 3 and 5 years being 2.11 (95% CI: 0.82–5.44), 1.87 (95% CI: 0.8–4.42), 2.07 (95% CI: 0.98–4.4) and 2.25 (95% CI: 1.09–4.66).

Table 3. Uni- and multivariate analysis of PTLD risk after first transplantation.

	Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Recipient gender				
Male	1 (reference)			
Female	0.99 (0.53–1.87)	0.978		
Age at transplantation, years				
0–17	4.15 (1.69–10.16)	0.002	0.35 (0.04–3.41)	0.365
18–35	2.19 (0.99–4.83)	0.053	1.58 (0.44–5.70)	0.482
36–53	1 (reference)		1 (reference)	
>53	0.92 (0.33–2.54)	0.873	1.38 (0.30–6.38)	0.684
Donor status				
Alive	1 (reference)		1 (reference)	
Deceased	0.46 (0.24–0.89)	0.022	0.48 (0.16–1.47)	0.200
Donor gender				
Male	1 (reference)		1 (reference)	
Female	0.49 (0.25–0.98)	0.044	0.51 (0.18–1.47)	0.212
Recipient blood type				
0	1 (reference)			
A	0.57 (0.29–1.11)	0.099		
B	1.05 (0.40–2.77)	0.924		
AB	0.34 (0.05–2.56)	0.298		
Degree of HLA mismatch: A, B, DR				
0	1 (reference)			
1–3	0.60 (0.23–1.59)	0.306		
4–6	0.42 (0.14–1.27)	0.126		
EBV serostatus before tx				
Recipient negative	1 (reference)		1 (reference)	
Recipient positive	0.03 (0.01–0.1)	<0.001	0.03 (0.01–0.1)	<0.001

PTLD risk

Univariate analysis (Table 3) showed the variables associated with risk of PTLD development to be living donor transplantation, male donor transplantation and recipient EBV seronegativity before transplantation. Age <18 years at transplantation was associated with a higher risk for PTLD compared with age 36–53 years. In the multivariate analysis, only EBV seronegativity of the recipient retained independent prognostic value.

Discussion

We investigated the incidence and presentation of PTLD after kidney transplantation over a 22-year period in a population-based cohort in western Denmark. Our study design with the Danish registration of all transplanted patients and a systematic review of all histological specimens from these patients give a valid and precise incidence. As all kidney transplant recipients are registered in the Danish Nephrology Registry, we were able to identify a complete population-based patient cohort. Furthermore, by linkage with the Danish National Pathology Registry, we could review all histology reports in these transplant recipients and, when relevant, retrieve the corresponding primary paraffin-embedded specimens for review. This strong design gives the study considerable advantages over most previously reported series, which we believe enabled us to identify the vast majority, if not all clinically relevant PTLDs in the cohort. Moreover, we found a rising incidence 10 years and later after transplantation; furthermore, it was shown that a fraction of the patients had multiple PTLDs, while 2 of 10 retransplanted PTLD patients subsequently had another PTLD.

We identified 70 PTLD cases, corresponding to an IR of 5.4 cases per 1000 Pt-yrs after first transplantation, including early lesions. In concordance with previous reports [5–7], we identified recipient EBV seronegativity at the time of transplantation to be the strongest risk factor for PTLD development. Moreover, we found a higher mortality among patients with PTLD compared with PTLD-free patients. Interestingly, death-censored graft survival was not inferior in patients with PTLD, pointing to the possibility of minimizing and adjusting immunosuppression in order to preserve the graft.

We found a higher total incidence of PTLD than is usually reported [11,12]. The cumulative incidence after 5 and 10 years was, however, comparable with some other studies [2,28,29]. While the high total incidence may reflect a number of factors, we believe that the meticulousness of our survey of all kidney transplant recipients in the cohort is the main explanation. This allowed a more complete identification of PTLD cases, including early lesions that are likely to be poorly registered in most cancer databases, as they mostly follow an indolent course, although we found both that relapse with malignancy does occur and that a case went unrecognized without progression. A search of the Danish Lymphoma Group Registry (that includes all patients with lymphoma treated at a Department of Haematology) revealed that none of the early lesions and only 61% of the other PTLD cases we identified had been registered. Moreover, the analysis also included children, who are known to have a higher incidence of PTLD, in part because of the high frequency of recipient EBV seronegativity at this age and thus an increased risk of developing primary EBV infection [1,7,16,30]. In support of this, IR for adult patients in the cohort was lower, being more similar to the incidence of PTLD reported by Morton *et al.* [12]. Finally, to avoid steroids, one of the centres previously used an immunosuppression regimen that

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included high-dose cyclosporine combined with induction with high-dose antithymocyte globulin. A high degree of immunosuppression, in particular T-cell function, has previously been identified as a risk factor for the development of PTLD [7]. The rather high incidence of early lesions found in our cohort was partly related to inclusion of children, as the majority of patients with early lesions were young at the time of transplantation.

Even though the study includes a meticulous review of possible PTLD, some cases may still have been undiscovered, for example because of missing biopsies or death without autopsy. Therefore, the IR we found is still only a minimum estimate of the true frequency. Another limitation of the study is the missing data, for example EBV serostatus before transplantation that was not measured as a matter of routine in the first years of the study period.

In agreement with other studies, we found the PTLD incidence to be highest in the first year after transplantation with a bimodal incidence pattern [2,12,30,31]. The high incidence 10 or more years after transplantation may reflect less intensive monitoring and less attention to PTLD so many years after transplantation, and accumulation of immunosuppression. This suggests the need for studies on minimization of immunosuppression, to avoid long-term comorbidity in transplant recipients. We also found that early-onset PTLDs were associated with only extranodal presentation and involvement of the graft. These outcomes are in agreement with the findings of Schober *et al.* [23]. The latter finding may reflect the development of donor origin PTLD in the graft [32,33]. The association between late-onset PTLDs and cyclosporine, and early-onset PTLDs and tacrolimus is probably related to changes in the immunosuppressive regimens during the follow-up period, and the fact that children were given tacrolimus before this was standard in adults. In contrast to van Leeuwen *et al.* [30] and Quinlan *et al.* [31], who found a greater proportion of late-onset cases to be extranodal, we found that late-onset-PTLDs were more often located in the lymph nodes compared with early-onset-PTLDs. This inconsistency supports the interpretation of PTLD as a diverse disease [23,31] that requires vigilance from physicians to recognize the various symptoms and findings. To understand PTLD development better, and to improve management, prospective registration of cases and management by agreed protocols is warranted.

Our study confirms that PTLD is a life-threatening disease with an increased mortality [11]. In agreement with De Biase *et al.*, [34] we found graft survival (non-censored for death) to be inferior 5 years after transplantation in patients with PTLD. However, non-death-censored graft survival ≤3 years after transplantation and death-censored graft survival were not significantly inferior in our cohort. Moreover, there was a trend towards better graft survival (death-censored) more than 1 year after transplantation. Our result could be related to early detection of PTLD, which allows minimization of immunosuppression at an individual level. Naturally, cure of PTLD has a higher priority than graft preservation. Moreover, most patients were probably treated with low-dose immunosuppression and thus avoided graft loss related to calcineurin inhibitor toxicity. The finding of reasonable graft survivals in patients with PTLD is important, as it stresses that graftectomy may only be indicated when PTLD is diagnosed together with overt graft rejection. The graft seems able to survive on a lower dose of immunosuppression in most patients with PTLD.

Our overall survival rates after PTLD diagnosis were similar or better compared with previously reported rates [15,16,18]. Although the size of the population involved makes it difficult to draw firm conclusions, a shorter therapeutic delay is probably more significant in PTLD compared with sporadic lymphoproliferative disorders in immunocompetent patients, as reduction in immunosuppression should be instituted immediately at the mere suspicion of PTLD, while the need for further investigation is evaluated. Collaborative protocols are required in this area, for example management with minimization of chemotherapy.

In conclusion, using registry data and pathology review we found a rather high PTLD incidence in western Denmark. Of the 10 patients with PTLD who were retransplanted, two had a second PTLD. Death-censored graft survival was not inferior in patients with PTLD compared with other renal transplant recipients, suggesting grafts can survive on minimal immunosuppression.

Authorship

EFM, MØV, Fd'A, SH-D and BJ: designed the study. EFM, MØV, JK, MBM, CS, CB, HCT, ES, SH-D and BJ: collected the data. KB and SH-D: reviewed the pathology. EFM and MØV: analysed the data. All authors wrote and approved the manuscript.

Funding

This study was funded by the Danish Cancer Society; the NEYE Foundation; the Danish Kidney Foundation; the Helen and Ejnar Bjoernow Foundation; the Frits,

Georg and Marie Cecilie Glud Scholarship; the Rigmor and Alex Trosborg Memorial Scholarship; and the Managing Director Jacob Madsen and wife Olga Madsen Foundation.

Acknowledgements

The authors want to thank Frank Pedersen and Kaj Anker Jørgensen from Scandia Transplant, James Heaf from Danish Society of Nephrology and Peter Brown from Danish Lymphoma Group Registry for providing data and Bo Bibby for statistical assistance.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Flow chart [a]2 patients had two diverse cases of PTLD.

Table S1 Snomed[a]-search in Patobank.

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