Pediatr Nephrol (2017) 32:1433–1442
DOI 10.1007/s00467-017-3627-2

ORIGINAL ARTICLE

Viral load of EBV DNAemia is a predictor of EBV-related post-transplant lymphoproliferative disorders in pediatric renal transplant recipients

Elisa Colombini[1] · Isabella Guzzo[1] · Federica Morolli[1] · Germana Longo[2] · Cristina Russo[3] · Alessandra Lombardi[4] · Pietro Merli[4] · Luisa Barzon[5] · Luisa Murer[2] · Simone Piga[6] · Marta Luisa Ciofi degli Atti[6] · Franco Locatelli[4,7] · Luca Dello Strologo[1]

Received: 20 March 2016 / Revised: 16 February 2017 / Accepted: 16 February 2017 / Published online: 9 March 2017

Abstract
Background Post-transplant lymphoproliferative disorder (PTLD) is a severe complication of solid organ transplantation that can be classified into two major subtypes, namely, early lesions and non-early lesions, based on histopathological findings. In the vast majority of cases, proliferating cells are B lymphocytes and, most frequently, proliferation is induced by Epstein–Barr virus (EBV) infection.
Methods The aim of our study was to evaluate the natural history of EBV infection and its possible evolution toward PTLD in a pediatric cohort of patients who received a renal transplant between January 2000 and December 2013. A total of 304 patients were evaluated for this study, of whom 103 tested seronegative for EBV at transplantation.
Results Following transplantation, 50 of the 103 seronegative patients (48.5%) developed a first EBV infection, based on the results of PCR assays for EBV DNA, with 19 of these patients ultimately reverting to the negative state (<3000 copies/μl). Among the 201 seropositive patients only 40 (19.9%) presented a reactivation of EBV. Non-early lesions PTLD was diagnosed in ten patients, and early lesions PTLD was diagnosed in five patients. In all cases a positive EBV viral load had been

✉ Luca Dello Strologo
luca.dellostrologo@opbg.net

Elisa Colombini
elisa.colombini@inwind.it

Isabella Guzzo
isabella.guzzo@opbg.net

Federica Morolli
federica.morolli@opbg.net

Germana Longo
longo.germana@libero.it

Cristina Russo
cristina.russo@opbg.net

Alessandra Lombardi
alessandra.lombardi@opbg.net

Pietro Merli
pietro.merli@opbg.net

Luisa Barzon
luisa.barzon@unipd.it

Luisa Murer
murer@pediatria.unipd.it

Simone Piga
simone.piga@opbg.net

Marta Luisa Ciofi degli Atti
marta.ciofidegliatti@opbg.net

Franco Locatelli
franco.locatelli@opbg.net

[1] Pediatric Nephrology and Renal Transplant Unit, Bambino Gesù Children's Hospital–IRCCS, Piazza S. Onofrio 4, 00165 Rome, Italy

[2] Pediatric Nephrology, Dialysis and Transplantation Unit, Department of Pediatrics, University of Padova, Padova, Italy

[3] Virology Unit, Bambino Gesù Children's Research Hospital–IRCCS, Rome, Italy

[4] Hematology and Oncology, Bambino Gesù Children's Research Hospital–RCCS, Rome, Italy

[5] Department of Molecular Medicine, University of Padua, Padua, Italy

[6] Unit of Clinical Epidemiology, Bambino Gesù Children's Research Hospital–IRCCS, Rome, Italy

[7] University of Pavia, Pavia, Italy

detected at some stage of the follow-up. Having a maximum peak of EBV viral load above the median value observed in the whole cohort (59,909.5 copies/μl) was a significant and independent predictor of non-early lesions PTLD and all PTLD onset.

Conclusions A high PCR EBV viral load is correlated with the probability of developing PTLD. The definition of a reliable marker is essential to identify patients more at risk of PTLD and to personalize the clinical approach to the single patient.

Keywords Epstein–Barr virus · Renal transplant · Post-transplant lymphoproliferative disorder · Children · Viral load

Introduction

Post-transplant lymphoproliferative disorder (PTLD) is a severe complication of solid organ transplantation that develops due to uncontrolled proliferation of lymphocytes within the context of post-transplant immunosuppression. In the vast majority of cases, the proliferating cells are B lymphocytes of recipient origin and, most frequently, proliferation is induced by Epstein–Barr virus (EBV) infection [1].

The revised World Health organization (WHO) classification of PTLD considers PTLD histopathology independently of time of onset since transplantation and categorizes the continuum of disease into plasma cell hyperplasia/early lesion PTLD, polymorphic PTLD (polyclonal or monoclonal), and monomorphic PTLD [2, 3]. The term "early lesions PTLD" refers to localized early lesions that show the first morphological changes in the spectrum of PTLDs. A distinctive morphologic feature of early lesions PTLD is exuberant lymphoid proliferation with preservation of normal tissue architecture despite the presence of a mass effect, such as enlarged lymph nodes or tonsils and/or adenoid hypertrophy. This is in contrast with the other subtypes of non-early PTLDs (polymorphic, monomorphic) in which the normal architecture of the involved tissue is partially or completely destroyed [3, 4].

The incidence of the disease is higher in children who experience primary EBV infection post-transplantation, whereas the influence of the cumulative burden of immunosuppression is less evident [5]. Past studies have indicated that the incidence of PTLD after kidney transplantation is lower than that occurring after intestinal, heart, lung, and liver transplantation, but the increasing number of young renal transplant recipients may account for a higher rate of EBV-seronegative individuals receiving transplantation and, thus, for an increased prevalence of EBV-associated PTLD [6].

The aim of our study was to describe the natural history of EBV infection and its possible evolution toward PTLD in a large cohort of pediatric renal transplant recipients.

Methods

Patient population and data collection

This was a retrospective cohort study in which all patients who received a renal transplant in two large transplantation centers in Italy [Bambino Gesù Children's Hospital (Center A); University of Padua, (Center B)] between January 2000 and December 2013 were eligible for entry. Patients were enrolled in the study if the results of at least two real-time PCR assays for EBV DNA per year were available following transplantation. Data on the results of the real-time EBV PCR assay and PTLD were retrospectively collected from clinical records.

EBV DNA was monitored in whole blood using the Artus real-time EBV TM PCR kit (Qiagen, Hamburg, Germany) (Center A) and an in house-developed real-time quantitative (q)PCR assay (Center B) [7] that targets the conserved EBV nuclear antigen 1 (*EBNA1* gene) region and the highly conserved *BLLF1* gene, respectively. Amplification cycles were run in a qPCR assay using ABI Prism 7900 HT Fast Real-time instruments (Applied Biosystems, Foster City, CA). The viral load was calculated from the slope and intercept of the standard curve, and results were expressed in copies per microliter. Good agreement between the two methods was demonstrated by inter-laboratory evaluations. Test proficiency was monitored by regular external quality assessments using Quality Control for Molecular Diagnostics (QCMD) programs. The analytical sensitivity (limit of detection) of both methods was 100 copies/ml.

The clinical cut-off for a positive PCR result for EBV DNA was arbitrarily set at 3000 copies/μl. A patient who tested positive for EBV DNA was subsequently evaluated at regular intervals for possible development of PTLD by means of abdominal scan (every 6 months) and chest X-ray (at least annually).

Patients were treated with induction treatment with basiliximab (two shots, on day 0 and day 4) or thymoglobulin (for 5–7 days post-transplantation, with the dosage adjusted to maintain a lymphocyte count of <200/mmc). As maintenance treatment, patients received calcineurin inhibitors (tacrolimus or cyclosporine) and steroids. Most patients were also treated with mycophenolate, or more rarely with azathioprine or mTOR inhibitors. Valganciclovir was provided as prophylaxis to all patients at high risk of developing cytomegalovirus (CMV) infection (CMV-positive donor vs. CMV-negative recipients) for the first 3 months after transplantation.

We categorized PTLDs according to the WHO subtype classification as localized early lesions (early lesions PTLD) or non-early lesions PTLD (polymorphic, monomorphic B-cell and other subtypes) that may disseminate independently from the time of occurrence since transplantation [2, 3].

Statistical analysis

Primary study outcomes were: (1) positive PCR test result for EBV following transplantation, defined as >3000 copies/μl, (2) onset of non-early lesions PLTD, and (3) onset of all PTLDs. Categorical variables were summarized using absolute frequencies and percentages, and continuous variables by the mean or median and range, as appropriate. To determine statistical differences between groups, we used the Chi-square test or Fischer's exact test for categorical variables and the *t* test or Mann–Whitney test for continuous variables.

Three multivariate Cox proportional hazard models were developed to assess independent predictors of the three primary study outcomes. Variables for which the *p* value was ≤0.20 in univariate analysis were included in the multivariate models (likelihood ratio test $p < 0.05$). Proportional hazards assumptions were respected, and C-concordance statistics were used to measure the model performance.

All statistical analyses were performed using STATA, Statistical Software: Release 13 (StataCorp LP, College Station, TX).

Results

In the period considered, 318 patients underwent renal transplantation in the two participating centers. Of these, 14 patients were excluded from the analysis due to incomplete clinical data.

The characteristics of the 304 patients included in the study (201 from Center A, transplanted between January 2000 and December 2013, and 103 from Center B, transplanted between January 2004 and December 2013) are shown in Table 1. Of these 304 patients, 31 were older than 18 years at transplantation. Median duration of follow-up was significantly longer in Center A than in Center B, and significantly more patients were treated with antiviral prophylaxis in Center B than in Center A. Also, induction therapy with thymoglobulin and maintenance therapy with tacrolimus were more often used in Center B than Center A. All other patients' characteristics were similar between the two participating centers (Table 1).

Data on donor EBV status before transplantation were available for 152 patients (50%), of whom 121 were positive (79.6%) and 31 were negative (20.4%) (Table 1).

At time of transplantation, 103 patients were seronegative for EBV (33.9%) (Fig. 1). Following the graft, 50 of these (48.5%) developed a first EBV infection after a median time of 8.4 months (range 7.0 days to 11.4 years). Upon detection of EBV infection, immunosuppression was reduced in all cases (withdrawal of mycophenolate in most cases) (data not shown). The PCR EBV assay results showed a reversion to <3000 copies/μl in 19 of these patients (38%) after a median time of 22.1 months (13 days to 84 months). Median maximum viral load was 337,443 (range 4214–99,470,000) copies/μl in those who maintained throughout the period of observation a positive viral load and 19,447 (range 1635–8,034,000) copies/μl in those who subsequently became negative ($p < 0.001$).

Among the 201 patients who were seropositive at time of transplantation, only 40 (19.9%) presented a reactivation of EBV, based on PCR assay values that exceeded the predetermined threshold of 3000 copies/μl (Fig. 1), after a median time of 4.48 months (range 1 day to 9.3 years); in 19 (47.5%) of these patients EBV DNA decreased to <3000 copies/μl after a median time of 10 months. Median maximum viral load was 119,840 (range 4214–9,578,000) copies/μl in those patients who maintained a positive viral load and 6314 (range 1635–224,000) copies/μl in those who became negative ($p = 0.001$).

All EBV-seropositive patients (EBV ≥3000 copies/μL) were monitored over time using EBV PCR assays. determinations. The frequency of EBV seropositivity in the group of children who reached a high viral load (≥59,909.5 copies/μl; mean determinations per year upon detection of high viral load: 1.4) was similar to that the group who did not (<59,909.5 copies/μl; mean determinations per year: 1.6) ($p = 0.166$).

Very few patients (only 5) were treated with rituximab; these patients received one or two doses (375 mg/sq.m) with the intent to lower the EBV viral load in the blood. In all patients, the EBV viral load dropped very quickly after the rituximab treatment, becoming undetectable, but after a short time (2–6 months) it rose again in four patients to levels comparable to those detected before treatment, with only one patient having a persistent reduction of EBV viral load. None of these five patients developed PTLD after a follow-up of 3, 24, 28, 50 and 60 months, respectively. Given the very small number of patients in this group, no conclusions the effect of rituximab can be drawn.

Five patients had a diagnosis of early lesions PTLD and were treated with rituximab and a reduction of immunosuppression. These patients are still being closely monitored, but after a long follow-up (5–9 years) none of them has yet developed an aggressive lymphoma (Table 2).

Non-early lesions PTLD was diagnosed in ten patients (3.3% of the whole cohort). In all cases, it occurred in patients in whom a positive EBV viral load was detected at some stage of their follow-up, even though one-half of the patients became negative at some stage of their follow-up and only one case occurred in this latter group of patients (Fig. 1).

Non-early lesions PTLD occurred in three of the 201 patients (1.5%) who were seropositive at transplantation

Table 1 Characteristics of patients included in the study, by center

Patient characteristics	Center A	Center B	Total	*p* value
Total number of patients enrolled in study	201	103	304	
Number of male patients enrolled	102 (50.8%)	64 (62.1%)	166 (54.6%)	0.059
Median age at transplantation (years)	13.3 (2.9–26.9)	11.0 (1.5–30.4)	12.8 (1.5–30.4)	0.242
Median duration of follow-up	6.1 years (15 days to 14.8 years)	4.1 years (2.0 days to 7.7 years)	5.4 years (2 days to 14.8 years)	<0.001
Early lesions PTLD	2 (1.0%)	3 (2.9%)	5 (1.6%)	0.341
EBV seropositive at transplantation	137 (68.2%)	64 (62.1%)	201 (66.1%)	0.294
EBV PCR positive after transplantation	63 (31.3%)	27 (26.2%)	90 (29.6%)	0.354
EBV-seropositive donor	73 (79.4%)	48 (80.0%)	121 (79.6%)	0.922
CMV seropositive at transplantation	95 (55.2%)	47 (46.1%)	142 (51.8%)	0.143
Anti-viral ganciclovir prophylaxis	58 (33.0%)	60 (58.8%)	118 (42.5%)	<0.001
Induction treatment				0.037
Basiliximab	192 (95.5%)	91 (89.2%)	283 (93.4%)	
Thymoglobulin	9 (4.5%)	11 (10.8%)	20 (6.6%)	
Maintenance treatment				<0.001
Cyclosporine	143 (73.3%)	19 (18.6%)	162 (54.6%)	
Tacrolimus	52 (26.7%)	83 (81.4%)	135 (45.5%)	
Transplant rejection	67 (36.8%)	33 (32.4%)	100 (35.2%)	0.450
Non-early lesions PTLD	7 (3.5%)	3 (2.9%)	10 (3.3%)	1.000

Values in table are given as the number of patients with the percentage in parenthesis or as the median with the range in parenthesis. Missing data are not included in the table

EBV, Epstein–Barr virus; PTLD, post-transplant lymphoproliferative disorder; CMV, cytomegalovirus

and in seven of the 103 patients (6.8%) who were seronegative at transplantation ($p = 0.035$) (Table 3). In all ten cases, in situ hybridization demonstrated EBV positivity in tissues.

Patients who were diagnosed with non-early lesions PTLD were referred to the hematology unit of the respective hospital. All of these patients were treated with a combination of chemotherapy and rituximab. The majority of patients recovered and kidney function was maintained. However, one patient died (10.0%) and two patients lost kidney function (20.0%).

The results of the multivariate Cox proportional hazard model investigating predictors of a positive PCR assay result for EBV following transplantation showed that seropositivity at transplantation and age of transplant recipient of >14 years were independently and significantly associated with a lower risk of EBV infection (Table 4).

Results of the multivariate models investigating predictors of non-early PLTDs and all PTLDs showed that having a maximum peak of EBV viral load above the median value observed in the whole cohort (59,909.5 copies/μl) was independently and significantly associated with higher risk of PTLD onset. In both models, center was also independently and significantly associated with the outcomes, even though this finding has no clear explanation and should be further investigated, while patient's age >14 years was a risk factor for non-early PTLDs (Tables 5, 6).

Discussion

Immunosuppressed patients may fail to develop an effective immune response against EBV. This may lead to a persistent infection, which may be responsible for the development of a PTLD, a well-known life-threatening complication of solid organ transplantation. Its prevalence depends on the type of transplanted organ, age of the patient, type and intensity of immunosuppression, and pre-transplantation EBV status [8, 9]. The highest incidence of PTLD occurs among pediatric recipients of heart, lung, and intestine (5–10%), while the incidence in pediatric liver and kidney recipients is 2.4 and 2–4%, respectively [1]. Intensity of immunosuppression may account for these differences [10]. The majority of cases of PTLD occur early, even within the first months after organ transplantation, i.e., during peak immunosuppression [10]. The risk of PTLD is higher in EBV-seronegative children who receive an organ from an EBV-seropositive donor [11].

Among the 304 patients enrolled in our study, the overall incidence of PTLD was consistent with that reported in the general literature [12] and, as expected, a naive status for EBV before transplantation was a high-risk factor for developing a steadily persistent high PCR EBV viral load. In total, 103 patients were seronegative at transplantation; of these 48% presented a first EBV infection, and in 62% of this latter group the viral load remained persistently detectable. These latter patients were therefore at high risk for PTLD. It is also likely

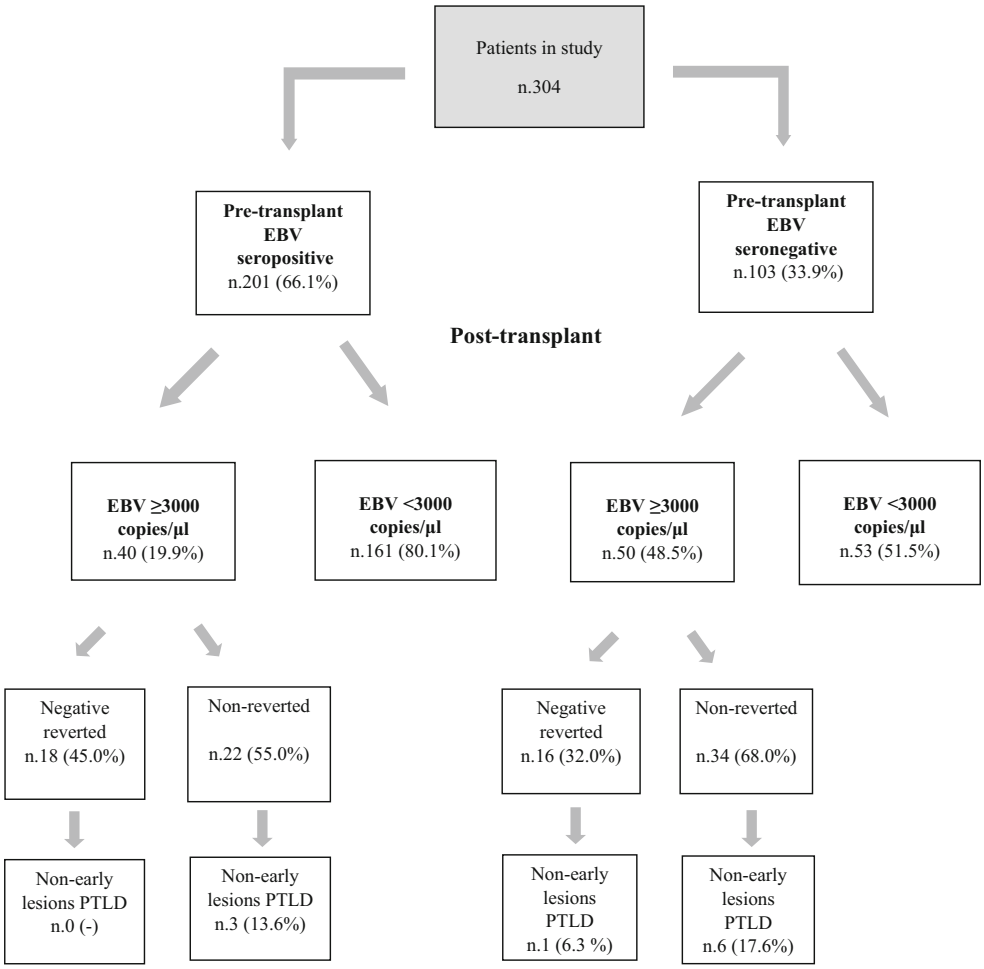

Fig. 1 Distribution of patients included in the study by Epstein–Barr virus (*EBV*) status and onset of non-early lesions post-transplant lymphoproliferative disorder (*PTLD*)

that the other seronegative patients will experience a first infection at some stage of their life. Hopefully, it will occur when immunosuppression is less aggressive, which might provide them with some advantages in controlling the infection.

With the introduction of serial viral load monitoring, it has become possible to identify a population of children who are at major risk of developing PTLD [13].

In our series all patients who developed a PTLD had a steady increase of EBV viremia. In our statistical model, a maximum peak of EBV viral load above the median value observed in the whole cohort (59,909.5 copies/µl) was significantly and independently associated with the development of a PTLD. This association remains an important point of debate in the literature: correlation with the maximum peak of EBV viral load has been demonstrated for various conditions, but it is not as evident for kidney transplant recipients [14–17].

Exposure to EBV begins early in life, with approximately 50% of children in developed countries becoming seropositive by 5 years of age [18]. The age of children at transplantation is therefore an indirect risk factor due to this higher likelihood of being seronegative in early infancy.

Table 2 Description of early lesions post-transplant lymphoproliferative disorder occurring in patients enrolled in the study

Patient	Age at transplant (years)	Pre-transplant EBV status	Early lesions PTLD		Outcome	
			Age at detection (years)	Site	Patient	Graft
1	2.6	Negative	11.7	Laterocervical lymph nodes	Remission	Functioning
2	3.1	Positive	4.0	Graft	Remission	Functioning
3	18.9	Negative	21.9	Laterocervical lymph nodes	Remission	Functioning
4	3.4	Negative	5.1	Laterocervical lymph nodes	Remission	Functioning
5	2.7	Negative	5.4	Tonsils	Remission	Functioning

Table 3 Description of post-transplant lymphoproliferative disorder occurring in patients enrolled in the study

Patient	Age at transplantation (years)	Pre-transplantation EBV status	Non-early lesions PTLD			Outcome	
			Age at detection (years)	Type	Site	Patient	Graft
1	3.9	Neg	16.0	Burkitt lymphoma	Abdominal lymph nodes	Deceased	Functioning
2	18.8	Pos	29.3	Large B cell lymphoma	Laterocervical lymph nodes	Remission	Functioning
3	17.1	Pos	23.0	Burkitt lymphoma	Laterocervical lymph nodes	On treatment	Functioning
4	15.7	Neg	21.0	Large B cell lymphoma	Laterocervical lymph nodes	Remission	Functioning
5	3.5	Neg	9.2	Large B cell lymphoma	Waldeyer's ring	Remission	Functioning
6	7.8	Neg	14.1	Large B cell lymphoma	Pretibial lymph nodes	On treatment	Lost
7	14.0	Pos	18.5	Large B cell lymphoma	Abdominal lymph nodes	On treatment	Functioning
8	15.8	Neg	16.2	Large B cell lymphoma	Laterocervical lymph nodes	Remission	Lost
9	21.2	Neg	22.5	Large B cell lymphoma	Laterocervical lymph nodes	Remission	Functioning
10	11.2	Neg	12.5	Large B cell lymphoma	Superior and inferior diaphragmatic	Remission	Functioning

Table 4 Predictors of positive PCR for Epstein–Barr virus. Multivariate Cox proportional hazard model

Predictors	Outcome: EBV >3000 copies/μl (n = 90 patients)								
	n	%	p	Univariate HR	95% CI	p	Multivariate HR	95% CI	p
Age of pediatric patient (years)			<0.001						
<10	49	54.4		1			1		
10–14	15	16.7		0.9	0.5–1.5	0.620	1.3	0.7–2.4	0.433
≥14	26	28.9		0.4	0.3–0.7	<0.001	0.6	0.3–0.9	0.044
Sex			0.971						
Female	41	45.6		1					
Male	49	54.4		0.9	0.6–1.5	0.930	NI	–	–
Hospital			0.354						
Center A	63	70.0		1			1		
Center B	27	30.0		1.9	1.2–3.2	0.011	1.7	0.9–3.03	0.061
Pre-transplant status			<0.001						
Seronegative	50	55.6		1			1		
Seropositive	40	44.4		0.3	0.2–0.4	<0.001	0.3	0.2–0.5	<0.001
EBV donor			0.281						
Seronegative	7	14.3		1					
Seropositive	42	85.7		1.3	0.6–2.8	0.557	NI	–	–
CMV at transplantation			0.354						
Seronegative	44	52.4		1					
Seropositive	40	47.6		0.9	0.6–1.4	0.587	NI	–	–
Induction treatment			0.297						
Basiliximab	82	91.1		1			1		
Thymoglobulin	8	8.9		1.8	0.86–3.7	0.119	2.5	1.2–5.3	0.020
Maintenance treatment			0.380						
Cyclosporine	52	58.4		1			1		
Tacrolimus	37	41.6		1.7	1.1–2.6	0.023	1.2	0.7–2.0	0.477
Anti-viral ganciclovir prophylaxis			0.694						
No	48	55.8		1					
Yes	38	44.2		1.1	0.7–1.6	0.800	NI	–	–

HR hazard ratio; CI, confidence interval; NI, variable that did not reach $p = 0.2$ in the univariate analysis and was not included in the final Cox model

Table 5 Predictors of non-early lesion post-transplant lymphoproliferative disorder onset. Multivariate Cox proportional hazard model

Predictors	Outcome: non-early lesions PTLD (n = 10 patients)								
	n	%	p	Univariate HR	95% CI	p	Multivariate HR	95% CI	p
Children's age (years)			0.193						
<10	3	30.0		1			1		
10–14	2	20.0		2.4	0.4–14.5	0.340	4.2	0.6–28.8	0.149
≥14	5	50.0		3.1	0.7–13.0	0.130	7.2	1.3–39.8	0.023
Sex			0.503						
Female	6	60.0		1					
Male	4	40.0		0.48	0.1–1.7	0.256	NI	–	–
Hospital			1.000						
Center A	7	70.0		1			1		
Center B	3	30.0		3.1	0.7–13.7	0.143	10.4	1.3–85.0	0.030
Pre-transplant status			0.502						
Seronegative	7	70.0		1					
Seropositive	3	30.0		0.5	0.1–2.0	0.314	NI	–	–
Maximum viral load (copies/μl)			0.015						
<59,909.5	1	10.0		1			1		
≥59,909.5	9	90.0		5.0	0.6–39.8	0.129	9.3	1.1–81.6	0.045
EBV donor			1.000						
Seronegative	0	–		1					
Seropositive	2	100.0		1.0	–	–	NI	–	–
CMV at transplantation			0.730						
Seronegative	4	44.4		1					
Seropositive	5	55.6		1.1	0.3–4.2	0.861	NI	–	–
Induction treatment			1.000						
Basiliximab	9	90.0		1					
Thymoglobulin	1	10.0		1.6	0.2–13.1	0.653	NI	–	–
Maintenance treatment			0.566						
Cyclosporine	5	50.0		1			1		
Tacrolimus	5	50.0		3.8	1.0–15.0	0.054	3.1	0.7–14.3	0.152
Anti-viral ganciclovir prophylaxis			0.694						
No	5	50.0		1					
Yes	5	50.0		1.4	0.4–4.8	0.620	NI	–	–

Consequently, transplanting young children carries a higher risk of PTLD development.

In our series, patient's age above 14 years also correlated with the development of non-early lesions PTLD. This finding deserves comment. Our study population was a pediatric cohort, and all patients had been placed on the waiting list for a transplant before the age of 18 years. However, transplantation may have occurred after 18 years of age and, as expected, almost all of them (97.8%) received a transplant before 22 years of age. Younger patients carry a higher probability of developing EBV infection and thus are at high risk for PTLD, but it is not clear why older patients should be at higher risk for non-early lesions PTLD. One possible explanation may be related to the worse adherence to treatment of adolescents. It has been demonstrated that patients in this age group are more at risk of rejection due to poor compliance [19–21], and it is possible that some of these patients may have received steroid pulses for rejection treatment which may have added to overall immunosuppression, inducing a higher risk for the development of non-early lesions PTLD.

Some authors have suggested a possible beneficial effect of valganciclovir in the treatment of EBV infection [22, 23], although the benefit of this drug has not been clearly demonstrated, as it acts only on replicating viruses, while in PTLD most of the viral genome is in a non-replicative phase [1]. In our series, CMV prophylaxis was provided for 3 months only to high-risk patients (positive donor/negative recipient). However, prophylaxis did not modify the risk for PTLD development in any of the statistical modeling.

Table 6 Predictors of all post-transplant lymphoproliferative disorder onset. Multivariate Cox proportional hazard model

Predictors	Outcome: all PTLD (n = 15 patients)								
	n	%	p	Univariate HR	95% CI	p	Multivariate HR	95% CI	p
Children's age (years)			0.636						
<10	7	46.7		1					
10–14	2	13.3		1.1	0.2–5.1	0.938			
≥14	6	40.0		1.6	0.5–4.9	0.383	NI	–	–
Sex			0.636						
Female	6	40.0		1					
Male	9	60.0		1.07	0.4–3.0	0.900	NI	–	–
Hospital			0.355						
Center A	9	60.0		1			1		
Center B	6	40.0		5.1	1.5–17.2	0.008	8.1	1.9–35.1	0.005
Pre-transplant status			0.161						
Seronegative	11	73.3		1			1		
Seropositive	4	26.7		0.4	0.1–1.3	0.132	0.6	0.2–1.9	0.370
Maximum viral load (copies/μl)			0.021						
<59,909.5	3	20.0		1			1		
≥59,909.5	12	80.0		2.2	0.6–8.0	0.218	4.3	1.1–17.5	0.044
EBV donor			0.554						
Seronegative	1	20.0		1					
Seropositive	4	80.0		0.6	0.1–5.4	0.648	NI	–	–
CMV at transplantation			0.845						
Seronegative	7	50.0		1					
Seropositive	7	50.0		0.9	0.3–2.6	0.869	NI	–	–
Induction treatment			1.000						
Basiliximab	14	93.3		1					
Thymoglobulin	1	6.67		1.0	0.1–7.8	0.994	NI	–	–
Maintenance treatment			0.892						
Cyclosporine	9	60.0		1			1		
Tacrolimus	6	40.0		2.4	0.8–7.3	0.131	1.2	0.3–4.3	0.753
Anti-viral ganciclovir prophylaxis			0.831						
No	8	53.3		1					
Yes	7	46.7		1.2	0.4–3.4	0.728	NI	–	–

Recent data from the literature [24] suggest that a possible alternative treatment of EV infection may be EBV-specific cytotoxic T lymphocytes. In our patient series, however, this approach was used only in exceptional cases, and there is therefore insufficient data to draw any conclusion on this efficacy of this treatment.

We must acknowledge several limitations to this study. It is retrospective, and we have included patients with different durations of follow-up. Some patients have a short follow-up and might have achieved a spontaneous recovery of EBV infection or recovered from the infection after immunosuppression reduction. Detection of a positive EBV PCR assay result often prompted physicians to increase the frequency of tests, thus EBV-positive patients may have had more determinations than the others.

There were a number of differences between the two centers: Center B used induction therapy with thymoglobulin and maintenance therapy with tacrolimus more often than Center A. Both induction and maintenance treatments were included in the multivariate model which investigated predictors of non-early lesions PTLD onset. Although thymoglobulin induction was significantly associated with EBV viremia, none of these covariates were significantly and independently associated with PTLD development. Thus, these variables do not explain the center effect in this model. This is an important issue that deserves further investigation in the future.

The most difficult issue is to predict which patients will actually develop PTLD, as not all patients with a persistent infection will develop lymphoma [25]. In our cohort, EBV maximum viral load significantly correlated with PTLD

development, and we should question if, in such a context, a sharp reduction of immunosuppression would have been appropriate [26], even at the risk of losing the kidney. In patients in whom PTLD occurred early after the detection of EBV viremia, any preventative measures would likely have been ineffective, while in those in whom the diagnosis of PTLD occurred several months or years after EBV detection, we would have had time initiate more effective treatment. In all cases immunosuppression was moderately reduced with little or no effect. However, it is important to consider that even among our high-risk patients (those with a persistently high EBV viral load) only a minority developed PTLD. Moreover, following the development of non-early lesions PTLD, the majority of patients recovered, although one of the ten patients who developed lymphoma died and two additional patients, who had recovered from PTLD, lost their graft. Stopping immunosuppression in all patients at risk is therefore inappropriate.

The definition of a reliable, more specific, marker of risk is essential to personalize the clinical approach to the single patient [27, 28].

Contribution of each author to the manuscript EC, IG, LM, and LDS participated in research design; EC, IG, LB, LM, FL, MCDA, and LDS participated in the writing of the paper; EC, FM, GL, CR, AL, and PM participated in carrying our the research, and SP, MCDA, EC, IG, LM, FL, and LDS participated in data analysis

Compliance with ethical standards

Ethics The study was approved by the OPBG Ethical Committee with a waiver of informed consent (study number: 1183-OPBG 2016).

Conflict of interest The authors declare no conflicts of interest.

References

1. Dharnidharka VR, Araya CE (2009) Post-transplant lymphoproliferative disease. Pediatr Nephrol 24:731–736
2. Glotz D, Chapman JR, Dharnidharka VR, Hanto DW, Castro MC, Hirsch HH, Leblond V, Mehta AK, Moulin B, Pagliuca A, Pascual J, Rickinson AB, Russo FP, Trappe RU, Webster AC, Zuckermann AO, Gross TG (2012) The Seville expert workshop for progress in posttransplant lymphoproliferative disorders. Transplantation 94: 784–793
3. Mucha K, Foroncewicz B, Ziarkiewicz-Wroblewska B, Krawczyk M, Lerut J, Paczek L (2010) Post-transplant lymphoproliferative disorder in view of the new WHO classification: a more rational approach to a protean disease? Nephrol Dial Transplant 25:2089–2098
4. Parker A, Bowles K, Bradley JA, Emery V, Featherstone C, Gupte G, Marcus R, Parameshwar J, Ramsay A, Newstead C (2010) Diagnosis of post-transplant lymphoproliferative disorder in solid organ transplant recipients—BCSH and BTS Guidelines. Br J Haematol 149:675–692
5. Allen UD, Farkas G, Hebert D, Weitzman S, Stephens D, Petric M, Tellier R, Ngan B, Fecteau A, West L, Wasfy S (2005) Risk factors for post-transplant lymphoproliferative disorder in pediatric patients: a case-control study. Pediatr Transplant 9:450–455
6. Sato T, Fujieda M, Tanaka E, Miyamura M, Chikamoto H, Hisano M, Akioka Y, Ishiura Y, Dohno S, Maeda A, Hattori M, Wakiguchi H (2008) Monitoring of Epstein–Barr virus load and antibody in pediatric renal transplant patients. Pediatr Int 50:454–458
7. Biasiolo A, Tono N, Zaninotto M, Merkel C, Fassina G, Plebani M, Gatta A, Pontisso P (2013) Specificity of squamous cell carcinoma antigen (SCCA)-IgM detection in patients with HCV infection and rheumatoid factor seropositivity. J Med Virol 85:1005–1008
8. Green M, Michaels MG (2013) Epstein-Barr virus infection and posttransplant lymphoproliferative disorder. Am J Transplant 13(Suppl 3):41–54
9. Allen UD, Preiksaitis JK (2013) Epstein–Barr virus and posttransplant lymphoproliferative disorder in solid organ transplantation. Am J Transplant 13[Suppl 4]:107–120
10. Wilde GE, Moore DJ, Bellah RD (2005) Posttransplantation lymphoproliferative disorder in pediatric recipients of solid organ transplants: timing and location of disease. AJR Am J Roentgenol 185: 1335–1341
11. Suzuki T, Ikezumi Y, Okubo S, Uchiyama M, Takahashi K, Shiraga H, Hattori M (2007) Epstein–Barr virus DNA load and seroconversion in pediatric renal transplantation with tacrolimus immunosuppression. Pediatr Transplant 11:749–754
12. McDonald RA, Smith JM, Ho M, Lindblad R, Ikle D, Grimm P, Wyatt R, Arar M, Liereman D, Bridges N, Harmon W (2008) Incidence of PTLD in pediatric renal transplant recipients receiving basiliximab, calcineurin inhibitor, sirolimus and steroids. Am J Transplant 8:984–989
13. Bingler MA, Feingold B, Miller SA, Quivers E, Michaels MG, Green M, Wadowsky RM, Rowe DT, Webber SA (2008) Chronic high Epstein–Barr viral load state and risk for late-onset posttransplant lymphoproliferative disease/lymphoma in children. Am J Transplant 8:442–445
14. Ishihara M, Tanaka E, Sato T, Chikamoto H, Hisano M, Akioka Y, Dohno S, Maeda A, Hattori M, Wakiguchi H, Fujieda M (2011) Epstein–Barr virus load for early detection of lymphoproliferative disorder in pediatric renal transplant recipients. Clin Nephrol 76: 40–48
15. Tanaka E, Sato T, Ishihara M, Tsutsumi Y, Hisano M, Chikamoto H, Akioka Y, Dohno S, Maeda A, Hattori M, Wakiguchi H, Fujieda M (2011) Asymptomatic high Epstein–Barr viral load carriage in pediatric renal transplant recipients. Pediatr Transplant 15:306–313
16. Fox CP, Burns D, Parker AN, Peggs KS, Harvey CM, Natarajan S, Marks DI, Jackson B, Chakupurakal G, Dennis M, Lim Z, Cook G, Carpenter B, Pettitt AR, Mathew S, Connelly-Smith L, Yin JA, Viskaduraki M, Chakraverty R, Orchard K, Shaw BE, Byrne JL, Brookes C, Craddock CF, Chaganti S (2014) EBV-associated post-transplant lymphoproliferative disorder following in vivo T-cell-depleted allogeneic transplantation: clinical features, viral load correlates and prognostic factors in the rituximab era. Bone Marrow Transplant 49:280–286
17. Ruf S, Behnke-Hall K, Gruhn B, Reiter A, Wagner HJ (2014) EBV load in whole blood correlates with LMP2 gene expression after pediatric heart transplantation or allogeneic hematopoietic stem cell transplantation. Transplantation 97:958–964
18. Cohen JI (2000) Epstein–Barr virus infection. N Engl J Med 343: 481–492
19. Van Arendonk KJ, King EA, Orandi BJ, James NT, Smith JM, Colombani PM, Magee JC, Segev DL (2015) Loss of pediatric kidney grafts during the "high-risk age window": insights from pediatric liver and simultaneous liver–kidney recipients. Am J Transplant 15:445–452
20. Van Arendonk KJ, James NT, Boyarsky BJ, Garonzik-Wang JM, Orandi BJ, Magee JC, Smith JM, Colombani PM, Segev DL (2013)

Age at graft loss after pediatric kidney transplantation: exploring the high-risk age window. Clin J Am Soc Nephrol 8:1019–1026
21. Van Arendonk KJ, James NT, Locke JE, Montgomery RA, Colombani PM, Segev DL (2011) Late graft loss among pediatric recipients of DCD kidneys. Clin J Am Soc Nephrol 6:2705–2711
22. Hocker B, Bohm S, Fickenscher H, Kusters U, Schnitzler P, Pohl M, John U, Kemper MJ, Fehrenbach H, Wigger M, Holder M, Schroder M, Feneberg R, Kopf-Shakib S, Tonshoff B (2012) (Val-)Ganciclovir prophylaxis reduces Epstein–Barr virus primary infection in pediatric renal transplantation. Transpl Int 25:723–731
23. Hierro L, Diez-Dorado R, Diaz C, De la Vega A, Frauca E, Camarena C, Munoz-Bartolo G, de Gonzalez ZA, Lopez SM, Jara P (2008) Efficacy and safety of valganciclovir in liver-transplanted children infected with Epstein–Barr virus. Liver Transpl 14:1185–1193
24. Ricciardelli I, Blundell MP, Brewin J, Thrasher A, Pule M, Amrolia PJ (2014) Towards gene therapy for EBV-associated posttransplant lymphoma with genetically modified EBV-specific cytotoxic T cells. Blood 124:2514–2522
25. Hocker B, Fickenscher H, Delecluse HJ, Bohm S, Kusters U, Schnitzler P, Pohl M, John U, Kemper MJ, Fehrenbach H, Wigger M, Holder M, Schroder M, Billing H, Fichtner A, Feneberg R, Sander A, Kopf-Shakib S, Susal C, Tonshoff B (2013) Epidemiology and morbidity of Epstein–Barr virus infection in pediatric renal transplant recipients: a multicenter, prospective study. Clin Infect Dis 56:84–92
26. Styczynski J, Gil L, Tridello G, Ljungman P, Donnelly JP, van der Velden W, Omar H, Martino R, Halkes C, Faraci M, Theunissen K, Kalwak K, Hubacek P, Sica S, Nozzoli C, Fagioli F, Matthes S, Diaz MA, Migliavacca M, Balduzzi A, Tomaszewska A, Camara RL, van Biezen A, Hoek J, Iacobelli S, Einsele H, Cesaro S (2013) Response to rituximab-based therapy and risk factor analysis in Epstein Barr Virus-related lymphoproliferative disorder after hematopoietic stem cell transplant in children and adults: a study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. Clin Infect Dis 57:794–802
27. Lustberg ME, Pelletier RP, Porcu P, Martin SI, Quinion CD, Geyer SM, Caligiuri MA, Baiocchi RA (2014) Human leukocyte antigen type and posttransplant lymphoproliferative disorder. Transplantation 99:1220–1225
28. Furukawa S, Wei L, Krams SM, Esquivel CO, Martinez OM (2013) PI3Kdelta inhibition augments the efficacy of rapamycin in suppressing proliferation of Epstein–Barr virus (EBV) + B cell lymphomas. Am J Transplant 13:2035–2043