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Post-Transplant Lymphoproliferative Diseases in Pediatric Kidney Allograft Recipients with Epstein-Barr Virus Viremia

Hyesun Hyun,[1] **Eujin Park**,[2] **Myunghyun Cho**,[3] **Sang-Il Min**,[4] **Jongwon Ha**,[4,5] **Hyoung Jin Kang**,[6] **Hee Young Shin**,[6] **Il-Soo Ha**,[3] **Hae Il Cheong**,[3] **Yo Han Ahn**,[7] **and Hee Gyung Kang**[3]

[1]Department of Pediatrics, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea
[2]Department of Pediatrics, Kangnam Sacred Heart Hospital, Hallym University College of Medicine, Seoul, Korea
[3]Department of Pediatrics, Seoul National University Children's Hospital, Seoul National University College of Medicine, Seoul, Korea
[4]Department of Surgery, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea
[5]Transplantation Research Institute, Seoul National University College of Medicine, Seoul, Korea
[6]Department of Pediatrics, Seoul National University College of Medicine, Seoul National University Cancer Research Institute, Seoul, Korea
[7]Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, Korea

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Address for Correspondence:
Hee Gyung Kang, MD, PhD
Department of Pediatrics, Seoul National University Children's Hospital, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea.
E-mail: kanghg@snu.ac.kr

Yo Han Ahn, MD
Department of Pediatrics, Seoul National University Bundang Hospital, 82 Gumi-ro 173-beon-gil, Bundang-gu, Seongnam 13620, Korea.
E-mail: yhahn@snub.org

ORCID iDs
Hyesun Hyun
https://orcid.org/0000-0001-8525-1471
Eujin Park
https://orcid.org/0000-0002-4413-468X
Myunghyun Cho
https://orcid.org/0000-0002-3237-3173
Sang-Il Min
https://orcid.org/0000-0002-0688-0278
Jongwon Ha
https://orcid.org/0000-0003-2285-3517
Hyoung Jin Kang
https://orcid.org/0000-0003-1009-6002

ABSTRACT

Background: Post-transplant lymphoproliferative disease (PTLD) is one of the major complications of organ transplantation, especially in children with Epstein-Barr virus (EBV) viremia (EV). We performed a retrospective study to evaluate risk factors for PTLD in children with EV.

Methods: Among 199 pediatric kidney transplantation (KT) recipients at our center from January 2001 to October 2015, records of those with EBV viral loads of > 1,000 copies/mL and/or PTLD were reviewed.

Results: Diagnosis of PTLD was made in seven patients (PTLD group), and 39 patients had EV only (EV only group). The median time from KT to EV and PTLD diagnosis was 6.7 (range 0.4–47.8) months and 8.2 (range, 2.8–98.9) months, respectively. There were no significant differences between the groups in terms of sex, age at transplantation, donor type, EBV viral load, or EV-free duration after KT. Higher tacrolimus level before EV (hazard ratio, 44.5; $P = 0.003$) was an independent risk factor for PTLD in multivariate Cox regression analysis. Six patients with a high EBV load (median 171,639 copies/mL) were treated with preemptive rituximab (RTX) therapy, resulting in transient reduction of EBV load. None of these patients developed PTLD (median follow-up 51.5 months); however, two had neutropenia and two developed infection requiring hospital admission.

Conclusion: In pediatric KT recipients, higher tacrolimus levels were associated with a higher incidence of PTLD. Conversely, those who received preemptive RTX for EV did not develop PTLD.

Keywords: Post-Transplant Lymphoproliferative Disease; Kidney Transplantation; Epstein-Barr Virus; Rituximab

Hee Young Shin
https://orcid.org/0000-0003-2091-1947
Il-Soo Ha
https://orcid.org/0000-0001-5428-6209
Hae Il Cheong
https://orcid.org/0000-0001-7556-1265
Yo Han Ahn
https://orcid.org/0000-0002-8185-4408
Hee Gyung Kang
https://orcid.org/0000-0001-8323-5320

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Disclosure

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Hyun H, Ahn YH, Kang HG. Data curation: Hyun H, Park E, Cho M, Min SI, Ha J, Kang HJ, Shin HY, Ha IS, Cheong HI, Ahn YH, Kang HG. Formal analysis: Hyun H, Ahn YH. Investigation: Hyun H, Park E, Cho M, Min SI, Ha J, Kang HJ, Shin HY, Ha IS, Cheong HI, Ahn YH, Kang HG. Methodology: Hyun H, Park E, Cho M, Ha IS, Cheong HI, Ahn YH, Kang HG. Validation: Min S, Ha J, Kang HJ, Shin HY. Writing - original draft: Hyun H, Kang HG. Writing - review & editing: Hyun H, Ahn YH, Kang HG.

INTRODUCTION

Post-transplant lymphoproliferative disease (PTLD) is one of the major complications of organ transplantation, with an incidence ranging from 1% to 16% depending on the allograft organ.[1] This incidence is 0.2%–2.5% in liver and kidney transplantation cases, which is relatively lower than that in other organ transplant cases.[2,3] Incidence rates of PTLD are higher in pediatric kidney transplant recipients than in adult kidney transplant recipients, ranging from 1.2% to 10%.[4-6] More than 90% of pediatric PTLDs are due to Epstein-Barr virus (EBV)-positive B-cell proliferation. Previously reported risk factors for EBV-associated PTLD include recipient EBV seronegativity, degree of immunosuppression, acute rejection episode, use of OKT3 or tacrolimus, recipient age and race, allograft type, host genetic variations, and especially Epstein-Barr virus viremia (EV).[7-12] While adult allograft recipients usually have acquired-immunity to EBV at the time of transplantation, pediatric allograft recipients often experience primary EBV infection after transplantation. Although EBV-infected transformed B cells are highly immunogenic and rapidly eliminated by EBV-specific T cells in healthy hosts, if immunosuppressed pediatric patients have primary EBV infection, then an inadequate immune response may result in massive infection of B cells. Primary EBV infection increases the chance of developing PTLD 6–76 fold.[13-16]

EBV infection and/or reactivation, which can be detected as increasing copy numbers of EBV DNA in the peripheral blood, usually precede PTLD. Therefore, it is recommended that EBV titer be regularly monitored after solid-organ transplantation in patients at a higher risk for PTLD. The Kidney Disease Improving Global Outcomes clinical practice guideline for the care of kidney transplant recipients recommended the following monitoring regimen for EBV viral load monitoring in EBV-seronegative patients who received an allograft from a seropositive donor: every 1 week in the first 3 months after transplantation, at least monthly for 3–6 months, and then every 3 months for the rest of the first year.[17] Once EBV infection and/or reactivation is noted, transplantation physicians reduce immunosuppression to prevent EBV-associated PTLD. However, EBV infection often persists and progresses despite reduction in immunosuppressive drug regimen, and there is no anti-viral agent with proven efficacy against EBV.[8] In contrast, during stem cell transplantation, pre-emptive treatment with rituximab is often used to eradicate B lymphocytes, the reservoir of EBV, thereby to prevent PTLD.[18,19] A similar approach has been attempted in solid organ transplantation in patients at high-risk for EBV-associated PTLD.[20]

In this study, the risk factors for PTLD were assessed in pediatric kidney allograft recipients with EV, including the effect of pre-emptive rituximab treatment to prevent PTLD.

METHODS

Patients and data collection

We performed a retrospective study that included all patients aged 0–19 years who underwent kidney transplantation in Seoul National University Children's Hospital from January 2001 to October 2015. Patients were enrolled if their EBV load in whole blood was greater than 1,000 copies/mL for 2 consecutive tests. The EBV Q-PCR Alert kit (ELITech Group, Puteaux, France) was used to quantify the amount of Epstein-Barr virus nuclear antigen (EBNA)-1 in whole blood. The diagnosis of PTLD was made histologically after biopsy, and the association with EBV was assessed in tissue specimens by in-situ hybridization of Epstein-Barr virus-encoded

RNA (EBER). Patients who received another solid organ transplantation before or after kidney transplantation were excluded. Data were obtained from electronic medical records. Data reviewed included underlying disease, sex, age at transplantation, EBV serologic status of the donor and the recipient at transplantation, donor source, type of induction therapy, maintenance immunosuppressive medication, rejection episodes, and tacrolimus level at EV onset and median levels before and after onset of EV. The median levels of tacrolimus before the onset of EV were calculated using all values measured from transplantation to the appearance of EV. EBV serostatus at the time of transplant was determined by viral capsid antigen (VCA)-IgM, VCA-IgG, and EBNA. Cytomegalovirus (CMV) viremia was defined as positive antigenemia or detection of CMV DNA determined by whole-blood PCR.

Immunosuppression

In our center, induction immunosuppression therapy for kidney transplantation consisted of methylprednisolone with or without basiliximab or antithymocyte globulin. Steroids, tacrolimus, and mycophenolate mofetil were started perioperatively and continued as maintenance therapy. Methylprednisolone was administered as 10 mg/kg intravenous bolus dose at the time of surgery and was tapered gradually to a maintenance dose of prednisolone 0.3 mg/kg by 1 month after transplantation. The target tacrolimus trough level was 8–12 ng/mL for up to 3 months, 6–8 ng/mL between 3 and 6 months, and 4–6 ng/mL thereafter. Tacrolimus levels were monitored weekly for a month after discharge from operation, biweekly for the next 3 months, and monthly thereafter. From 2001 to 2008, basiliximab was used as induction therapy for high-risk patients with deceased-donor kidney transplant or higher number of human leukocyte antigen (HLA) mismatches. After 2008, most patients received basiliximab induction therapy.

EV monitoring

Principally, EBV was monitored every month for the first three months following transplant, then every 3 months for the rest of the first year, and then yearly. For recipients who were positive for EBV VCA IgG, EBV was monitored every three months. When EV was detected, EBV was monitored again in 2 weeks, and if the titer was still high, immunosuppression was reduced, usually by reducing antimetabolites and then tacrolimus according to the judgment of the physicians.

Pre-emptive rituximab therapy

Some of the patients who exhibited persistent high titers of EV (more than 1×10^4 copies/mL in whole blood for two consecutive weeks) despite immunosuppression reduction were treated with rituximab (RTX). These patients had prolonged high viremia over 1 year, a 3-fold or greater increase in EBV titer, or higher risk of malignancy (*WT1* mutation). After confirming that the patients did not have active infection or neutropenia, a single dose of RTX therapy of 375 mg/m^2 body surface area was administered.

Statistical analysis

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. Cox regression analysis was performed to identify risk factors for PTLD following EV. We performed the univariate Cox regression test to identify significant independent variables, and used independent variables with a univariate *P* value < 0.2 for multivariate Cox regression analysis. A *P* value < 0.05 was considered statistically significant. The statistical analysis was performed using IBM SPSS Statistics version 22.0 (IBM cooperation, Armonk, NY, USA).

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Ethics statement

The study was approved by the Institutional Review Board (IRB) of our center (IRB No. H-1312-068-541). The informed consent requirement was waived by the board.

RESULTS

During the study period, 199 children underwent kidney transplantation in our center. Of these, 46 (23.1%) had viremia defined as an EBV load greater than 1,000 copies/mL in whole blood for 2 consecutive tests during a median follow-up period of 5.3 years (**Fig. 1**). Viremia of all patients (EBV > 1,000 copies/mL) was first detected at a median of 6.7 months (range, 0.4–47.8 months) after kidney transplantation. The diagnosis of PTLD was made in seven patients (PTLD group) at a median of 8.2 months (2.8–98.9 months) after transplantation. The other 39 patients had EV only (EV only group).

Clinical course of PTLD

Patients with PTLD presented with fever, lymph node enlargement, or gastrointestinal symptoms such as abdominal pain, vomiting, and diarrhea (**Table 1**). Any gastrointestinal symptoms and/or lymph node enlargement raised suspicion for PTLD and prompted the clinician to perform further work-up to rule out PTLD. The majority of patients (n = 4) had gastrointestinal organ involvement, including small bowel and intraperitoneal lymph nodes. There was no extranodal PTLD. Pathologic diagnosis of PTLD revealed one case of early lesion, two cases of polymorphic PTLD, one case of Burkitt lymphoma, and three cases of diffuse large B-cell lymphoma. Upon diagnosis of PTLD, immunosuppressive medications

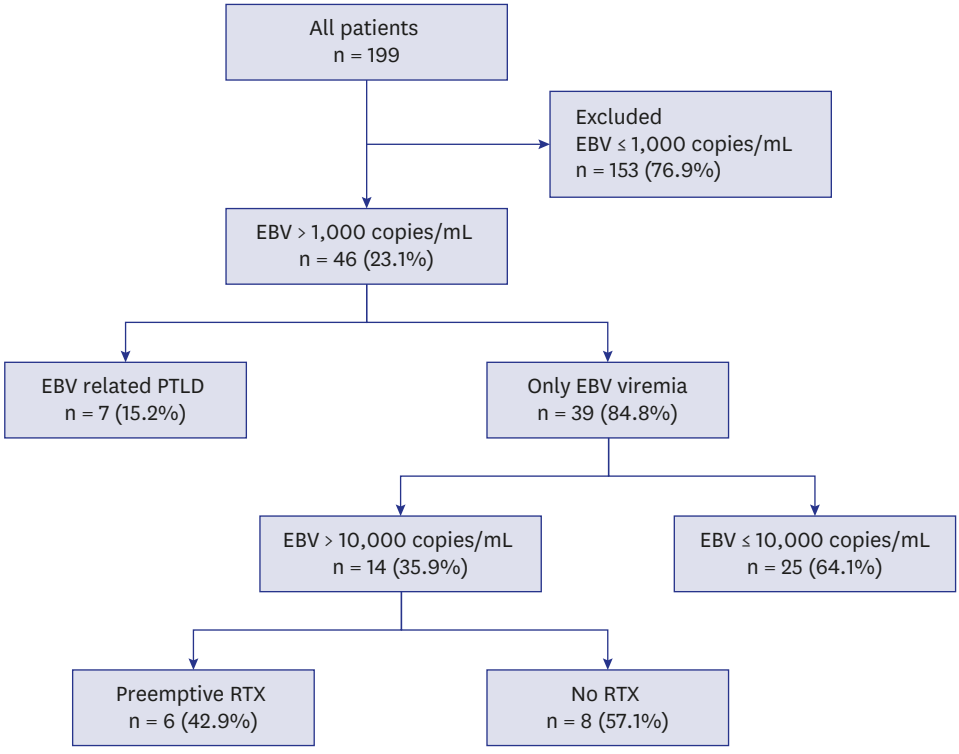

Fig. 1. Distribution of patients with kidney transplantation by EBV status and PTLD.EBV = Epstein-Barr virus, PTLD = post-transplant lymphoproliferative disease, RTX = rituximab.

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Table 1. Characteristics of patients with post-transplant lymphoproliferative disease

Case No.	Sex/age at time of PTLD, yr	Primary disease	Time from KT to PTLD, mon	Presentation	Involved site[a]	Histologic diagnosis	EBV serostatus	Treatment	EBV titer, copies/mL		Outcome	
									Peak	Median	PTLD	Graft
1	F/5	FSGS	10	Fever, cervical LNE	Neck mass, jugular LNs, portocaval LN	Diffuse large B-cell lymphoma[b]	D^{UK}/R^{UK}	RTX, chemotherapy	81,700	1,385	Remission	Function
2	F/7	FSGS	6	Vomiting, abdominal pain	Cervical, axillary, inguinal LNs, stomach, small bowel, umbilicus, vagina	Diffuse large B-cell lymphoma[b]	D^{UK}/R^{-}	RTX, chemotherapy	166,571	587	Remission	Function
3	F/14	FSGS	5	Epigastric pain, poor oral intake	Stomach, duodenum, mesenteric nodules	Diffuse large B-cell lymphoma[b]	D^{+}/R^{-}	RTX, chemotherapy	152,987	1,133	Remission	Lost
4	M/18	FSGS	3	Head and neck LNE	Cervical LNs	Polymorphic type[b]	D^{UK}/R^{-}	RTX	555	555	Remission	Function
5	F/7	Congenital NS	8	Tonsillar hypertrophy	Bilateral tonsils, cervical LNs	Early lesion[b]	D^{UK}/R^{-}	RTX	52,135	6,081	Remission	Function
6	M/4	Bilateral MCDK	10	Fever, cervical LNE	Cervical LNs	Polymorphic type[b]	D^{+}/R^{-}	RTX	134,159	208,947	Remission	Function
7	F/15	Fraiser syndrome[c]	99	Diarrhea, abdominal mass with tenderness	Perigastric, mesenteric LNs, stomach, small bowel	Burkitt lymphoma[b]	D^{UK}/R^{-}	RTX, chemotherapy	315,080	86,642	Remission	Function

PTLD = post-transplant lymphoproliferative disease, KT = kidney transplantation, EBV = Epstein-Barr virus, F = female, FSGS = focal segmental glomerulosclerosis, LNE = lymph node enlargement, LN = lymph node, D/R = donor/recipient, UK = unknown, RTX = rituximab, M = male, NS = nephrotic syndrome, MCDK = multicystic dysplastic kidney.
[a]Lesion detected by imaging study (computed tomography or positron emission tomography); [b]EBV in-situ hybridization positive; [c]Genetic disorder caused by *WT1* mutation.

were reduced, and RTX and/or chemotherapy were administered as appropriate. All patients achieved complete remission of PTLD after treatments. While one patient lost her allograft kidney due to complications of chemotherapy, six patients retained renal function after follow-up for 2.5–10.5 years.

Risk factors for PTLD

Table 2 shows the comparison of clinical variables between the PTLD and EV only group by univariate analysis. There were no significant differences between the two groups in terms of sex, age at transplantation, donor type, interval between transplantation, and first appearance of EV. Although the peak median EBV titer was higher in the PTLD group (152,987 EBV copies/mL whole blood) than the EV only group (17,305 copies/mL whole blood), there was no statistical significance. There were also no significant differences between groups in terms of median EBV viral load and EV-free duration after kidney transplant. At the time of transplantation, six patients (85.7%) in the PTLD group and 14 patients (35.9%) in the EV only group were seronegative for EBV ($P = 0.009$). Data of donor EBV status before transplantation were available only in a few cases, with no statistically significant difference observed between the groups.

Tacrolimus levels before EV tended to be higher in the PTLD group (9.5 ng/mL) than in the EV only group (7.7 ng/mL, $P = 0.039$). Maintenance immunosuppression regimen or history of rejection was not significantly different between the two groups. Six patients were treated with pre-emptive RTX, none of whom developed PTLD, while the number of RTX-treated patients was too small to be statistically significant.

The Cox proportional-hazard model was used to identify factors associated with an increased risk of developing PTLD after EV (**Table 3**). Values of 8.9 ng/mL for tacrolimus level and 35,900 copies/μL for peak EBV titer were determined as cutoff values based on the receiver operating characteristic curve analysis. The areas under curve of tacrolimus and peak EBV titer were 0.745 (95% confidence interval [CI] 0.522–0.969, sensitivity 85.7%, and specificity 79.5%) and 0.634 (95% CI 0.399–0.869, sensitivity 85.7%, and specificity

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Table 2. Characteristics of patients

Characteristics	PTLD (n = 7)	EV only (n = 39)	*P* value
Sex, M:F	2:5	18:21	0.446
Age at transplantation, yr	6 (3–18)	7 (1–16)	0.811
Donor type			1.000
Deceased	4 (57.1)	21 (53.8)	
Living related	3 (42.9)	18 (46.2)	
EBV recipient serostatus			0.009
Positive	0	23 (59.0)	
Negative	6 (85.7)	14 (35.9)	
Unknown	1 (14.3)	2 (5.1)	
EBV donor serostatus			1.000
Positive	2 (28.6)	10 (25.6)	
Negative	0	3 (7.7)	
Unknown	5 (71.4)	26 (66.7)	
Time to EV, mon	5 (1–48)	8 (0–47)	0.946
Peak EBV level, copies/mL	152,987 (555–1,341,159)	17,305 (1,198–1,279,841)	0.278
Median EBV level, copies/mL	6,081 (555–208,947)	4,250 (485–326,880)	0.834
CMV viremia	2 (28.6)	12 (30.8)	1.000
Induction therapy			
Basiliximab	6 (85.7)	22 (56.4)	0.220
Thymoglobulin	0	2 (5)	1.000
Maintenance medication			0.496
Steroid + tacrolimus + MMF	6 (85.7)	36 (92.3)	
Steroid + tacrolimus + AZA	1 (14.3)	1 (2.6)	
Steroid + tacrolimus	0	2 (5.1)	
Immunosuppressant after EV			0.423
Monotherapy	2 (28.6)	5 (12.8)	
Double immunotherapy	3 (42.9)	26 (66.7)	
Triple immunotherapy	2 (28.6)	8 (20.5)	
Tacrolimus level, ng/mL			
Pre EV diagnosis	9.5 (6.2–10.3)	7.7 (5.4–12.7)	0.039
At EV diagnosis	6.5 (2.5–9.6)	5.9 (2.2–14.3)	0.549
Post EV diagnosis	4.1 (0–5.8)	4.5 (2.4–7.2)	0.278
Rejection history	5 (71.4)	15 (38.5)	0.213
Preemptive RTX	0	6 (15.4)	0.266

Values are expressed as numbers (%) and median (range).
PTLD = post-transplant lymphoproliferative disease, EV = Epstein-Barr virus viremia, EBV = Epstein-Barr virus, CMV = cytomegalovirus, MMF = mycophenolate, AZA = azathioprine, RTX = rituximab.

59.0%), respectively. A higher tacrolimus level (hazard ratio [HR], 13.7; 95% CI, 1.6–117.9; $P = 0.017$) was associated with PTLD. Basiliximab induction therapy, higher EBV titer, EBV seronegativity of recipients, and rejection history were not significant in multivariate Cox regression analysis.

Table 3. Risk factors for post-transplant lymphoproliferative disease in patients with EV

Variables	Univariate			Multivariate[a]		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Tacrolimus level ≥ 8.9 ng/mL before EV	16.783	2.013–139.950	0.009	13.737	1.601–117.892	0.017
Basiliximab induction therapy	4.480	0.537–37.374	0.166	-	-	0.438
Peak EBV titer ≥ 35,900 copies/μL	7.304	0.877–60.832	0.066	-	-	0.149
Recipient EBV serostatus negative	87.887	0.112–69,135.034	0.188	-	-	0.054
Rejection history	3.651	0.707–18.852	0.122	-	-	0.285
Age, yr						
< 5	Reference					
6 to < 12	0.580	0.097–3.471	0.550	-	-	-
≥ 12	1.518	0.213–10.788	0.677	-	-	-

HR = hazard ratio, CI = confidence interval, EV = Epstein-Barr virus viremia, EBV = Epstein-Barr virus.
[a]Factors with a value of $P < 0.2$ in the univariate Cox regression analysis were included in the multivariate analysis.

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Table 4. Description of six patients with preemptive rituximab therapy

Patient No.	Age at KT, yr	Sex	Underlying disease	EBV serostatus	Duration of EV before RTX, mon	Median EBV before RTX, copies/mL	EBV at RTX, copies/mL	IS at RTX	EBV at last F/U, copies/mL	Rejection	Outcome
1	14	F	Lupus nephritis	D^{UK}/R^{-}	3.3	335,728	783,504	TAC, steroid	172,839	Acute rejection	ESRD
2	1	F	Denys-Drash syndrome[a]	D^{+}/R^{-}	1.2	266,825	266,825	TAC	192,321	No	Normal
3	10	M	Frasier syndrome[a]	D^{+}/R^{+}	18.3	20,296	283,074	TAC, sirolimus	2,050	No	Normal
4	2	F	Nephronophthisis	D^{UK}/R^{+}	46.1	19,738	26,464	TAC, sirolimus	1,122	No	CKD stage 2
5	2	F	Nephronophthisis	D^{+}/R^{-}	26.0	195,724	76,453	TAC, sirolimus	10,411	No	Normal
6	6	M	Acute tubular necrosis	D^{UK}/R^{-}	63.6	23,813	6,181	TAC, sirolimus	981	No	Normal

KT = kidney transplantation, EBV = Epstein-Barr virus, EV = Epstein-Barr virus viremia, RTX = rituximab, IS = immunosuppressant, F/U = follow up, F = female, D/R = donor/recipient, UK = unknown, TAC = tacrolimus, ESRD = end stage renal disease, CKD = chronic kidney disease, M = male.
[a]Genetic disorder caused by *WT1* mutation.

Efficacy and safety of pre-emptive rituximab treatment in pediatric kidney transplant

Six patients in the EV only group (male: female, 2:4) who had a high EBV load received preemptive RTX therapy. Their median age at transplant was 4 years (1–14 years), and EBV infection was first detected at 4.3 months (3.9–9.9 months) after kidney transplantation. Administration of RTX was carried out at a median of 29.2 months (5.1–69.6 months) after transplantation. These six patients did not exhibit any symptoms such as fever, lymph node enlargement, or gastrointestinal problems; and no imaging studies were performed. Two patients were EBV seropositive and four patients were EBV seronegative (**Table 4**). The two EBV-seropositive patients were at low risk for the development of PTLD, but RTX was administered to these patients based on the clinician's decision; one patient had a mutation of *WT1*, and was therefore prone to tumor development, while the other had persistently high EBV load for 46 months despite the reduction of immunosuppression. Median EBV viral loads at the time of RTX treatment were 171,639 copies/mL (6,181–783,504 copies/mL). After a single dose of RTX therapy, a concordant decrease in EBV load and B lymphocytes was observed (**Fig. 2**). In five patients, EV disappeared within months; the other patient showed reduction of EBV titer but persistence of EV despite RTX treatment. Unfortunately, the patient lost the allograft due to rejection and concomitant infection within 8 months after RTX therapy, and EBV titer was not monitored after this adverse event. In the remaining five patients, EBV load rebounded along with recovery of B cells in a median 8 months. However, none of these five patients developed PTLD over a median follow-up of 51.5 months.

Regarding the safety of RTX therapy, only one patient complained of chest discomfort during RTX infusion. Two patients experienced neutropenia at 4 months and 1 month after RTX treatment (**Fig. 3**). Two patients were admitted for viral or bacterial infections at 1 month and 2 months after RTX treatment. In comparison, of 8 patients with high EV (EBV > 10,000 copies/mL) but without RTX treatment, three patients experienced 6 infection episodes and one patient developed neutropenia during 12 months after the occurrence of high EV. Infectious complications included *Citrobacter freundii*, CMV, influenza A, adenovirus and *Pneumocystis jirovecii* infections. There were no significant differences in the infectious complications and neutropenia between the RTX group and the non-RTX group in patients with high EV.

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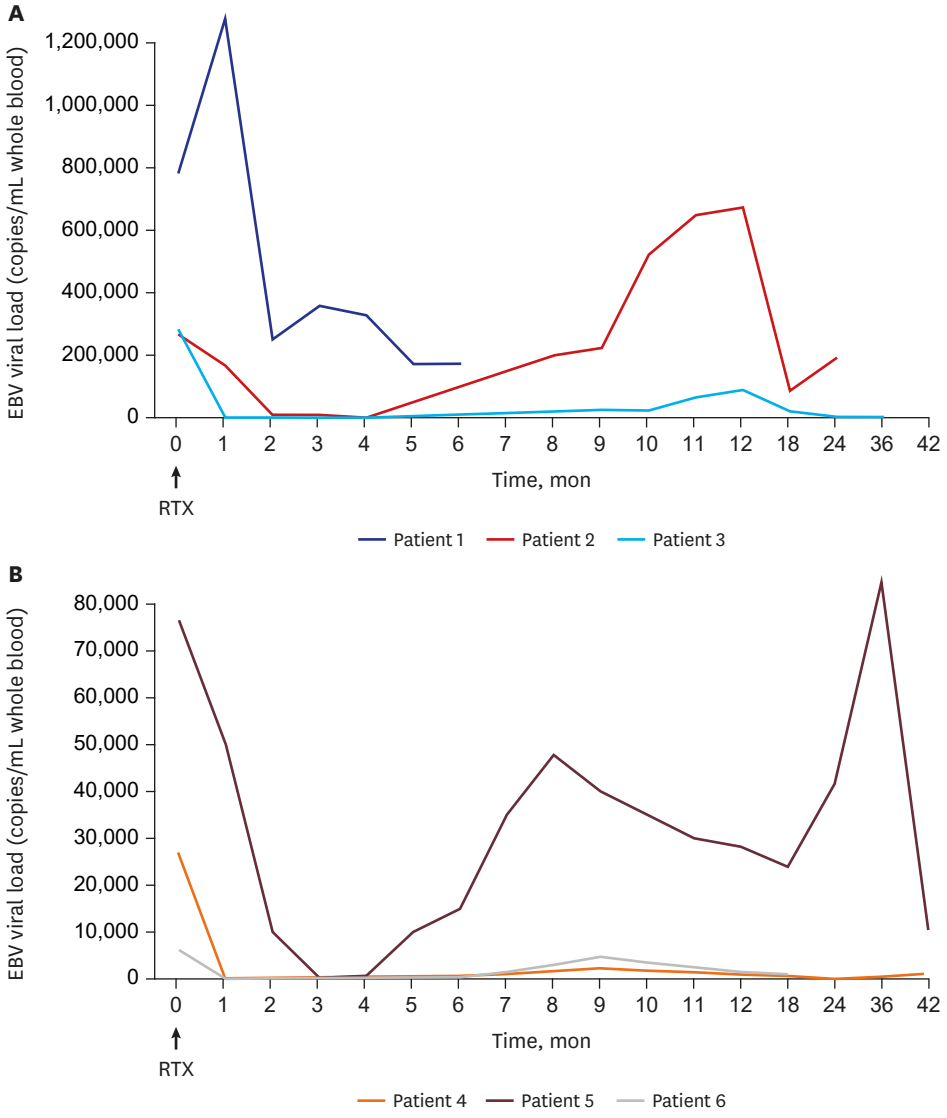

Fig. 2. EBV viral load after RTX therapy. (**A**) EBV viral loads in Patients 1, 2, and 3. (**B**) EBV viral loads in Patients 4, 5, and 6.
EBV = Epstein-Barr virus, RTX = rituximab.

DISCUSSION

During the last 15 years, among the almost 200 pediatric kidney transplantation recipients at our center, seven developed EBV-associated PTLD (3.5%). In our study, the risk factor for PTLD in pediatric kidney transplant recipients with EV was a high tacrolimus level before EV. Twenty (43.4%) out of 46 recipients who had viremia were EBV seronegative, which is similar to the rates reported in previous studies in North America and Europe (19%–57%).[21-23] Pre-transplant recipient EBV seronegativity is a well-known risk factor for PTLD. In adult transplant studies, the rate of developing PTLD is 5–12 fold higher in EBV-seronegative patients than in EBV-seropositive patients.[2,3,24] McDonald et al.[6] reported that EBV-seronegative pediatric subjects have a 4.7-fold higher relative HR than EBV-positive subjects. In our study, recipient EBV seronegativity did not increase the risk of PTLD in multivariate Cox regression analysis. While transplant from an EBV-seropositive donor to a seronegative recipient has been

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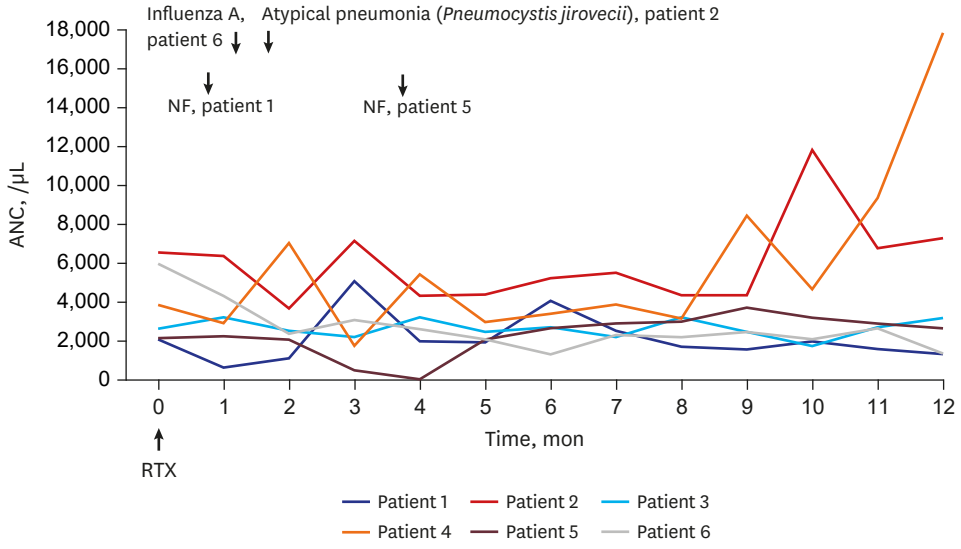

Fig. 3. Late adverse events after rituximab treatment.
ANC = absolute neutrophil count, NF = neutropenic fever, RTX = rituximab.

associated with the development of PTLD,[25] there was no statistically significant difference in EBV serostatus (donor/recipient) in the present study. This finding was attributed mainly to the fact that there were no EBV seropositive recipients in PTLD group and we did not have serostatus information for the majority of donors (67.3%).

The majority of kidney allograft recipients in this study were given tacrolimus, and mycophenolate was used in more than 80% of patients instead of azathioprine.[26] By the late 2000s, monoclonal interleukin-2 receptor antibodies were used as induction therapy in up to 80% of patients. Basiliximab, a monoclonal antibody which targets activated T lymphocytes, was not related to PTLD risk in our study, as previously reported.[4,27] Several studies have suggested that higher tacrolimus levels are associated with higher risk for PTLD, and others have reported that the net state of immunosuppression, rather than any individual agent, increases the risk for PTLD.[28-30] In our study, all patients received tacrolimus for maintenance immunosuppression, and we found that a higher pre-EV tacrolimus level in the PTLD group compared with the EV only group was a risk factor for PTLD.

Regular monitoring of EBV viral load and early recognition of recipients at high risk of PTLD have been identified as clinical priorities in recent years.[31] Previous studies have shown that elevated levels of EBV DNA and persistent high EBV loads are risk factors for PTLD,[12,20,32] but no clear cut-off point of EBV viral load for the prediction of PTLD development has been determined. We did not find a significant relationship between EV and PTLD in this study; however, six patients with a high EBV titer were treated with preemptive RTX and they did not develop PTLD. Because they were included in analysis, this could have confounded the causality of high EBV titer and PTLD development.

Treatment strategies for organ transplant recipients with EV include reduction of immunosuppression with/without antiviral agents, immunoglobulin, or RTX.[33] These treatments are still undergoing clinical studies. Preemptive administration of RTX is widely used and has been demonstrated to reduce the incidence of PTLD in stem cell transplant recipients with a high EBV viral load.[18,34,35] Pre-emptive RTX therapy has been reported in 14.5% of global

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transplant programs, and in more than 60% of pediatric transplant patients worldwide.[33] However, only one study reported the use of RTX in five pediatric renal allograft recipients,[20] and there have been no prospective studies on the efficacy of pre-emptive RTX therapy in solid organ transplantation. Rituximab is a murine/human chimeric monoclonal anti-CD20 antibody and is able to deplete circulating B cells rapidly, including those infected with EBV. Although RTX was effective for reducing EBV viral load in our patients, this reduction was not permanent in line with previous reports.[20] In addition, significant adverse effects, such as infection and neutropenia, accompanied RTX administration. In patients with stem cell transplant (SCT), preemptive RTX was not associated with an increase in infectious complications.[18,19] This is important because, while SCT recipients are able to discontinue their immunosuppressive agents within 6–9 months after SCT, recipients of solid organ transplantation have to be on immunosuppressive agents for as long as their allografts are functioning. Therefore, the long-term use of immunosuppressive agents may explain why infection and neutropenia are common clinical findings after RTX therapy in this patient population.

Although the small sample size of the current study precludes us from drawing any definite conclusions, our observations suggest that preemptive RTX treatment may effectively reduce high EBV viral load in pediatric recipients of solid organ transplants. Because RTX therapy eradicates B-lymphocytes including transformed lymphocytes, the risk of PTLD might be reduced at least during the period of B cell depletion. However, one should take into account that this treatment significantly increases the risk of neutropenia and infection. More research into the influence of preemptive RTX therapy on PTLD development is needed.

The occurrence of PTLD in kidney transplant recipients follows a bimodal distribution, with one peak in the first year and the second in the later post-transplantation period. Early PTLD, occurring within the first year of transplantation, is associated with EBV infection and tends to occur more commonly in children than adults.[12,36,37] In this study, while six patients in the PTLD group were diagnosed with PTLD within the first year of renal transplantation, one patient with *WT1* mutation developed PTLD later than 8.2 years after renal transplantation. We suspect that in this patient, the *WT1* mutation of a tumor suppressor gene might have increased the risk of PTLD, and especially that of late-onset. The occurrence of PTLD in patients with *WT1* mutation has been reported previously, within the first year in two cases and later than the first year in another.[38-40] Based on the limited availability of clinical data, the association between *WT1* mutation and development of PTLD requires further investigation in future clinical studies and is beyond the scope of the current study.

There are several limitations of this study. First, this is a retrospective observational study of a single center and the number of the patients observed was therefore small. The limitations of this small sample size might have affected the outcome of multivariate analysis. In addition, data of donor EBV serology were not uniformly available in our study. Data on donor serology status was only available for 33% of participants. Therefore, our study did not show any association between PTLD and EBV-donor/recipient serostatus. Finally, the number of patients who were treated with RTX was not large enough to draw any definitive conclusions.

In summary, this study demonstrates that a higher tacrolimus level before EV is correlated with the development of PTLD. Preemptive RTX appears to be effective for reducing EBV viral load in pediatric kidney transplant recipients. However, the reduction of EBV viral load was not persistent, and adverse effects of RTX, namely infection and neutropenia, were clinically significant.

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