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CLINICAL STUDY

Epstein–Barr virus infection in children with renal transplantation: 17 years experience at a single center

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Abstract

Objectives: The aim of this study was to detect the frequency, time of occurrence, management and outcome of Epstein–Barr virus (EBV) infection and related complications in pediatric renal transplant recipients. *Methods*: Pediatric renal allograft recipients transplanted between August 1994 and December 2011 at our hospital was evaluated retrospectively. The patients were divided into two groups; Groups 1 and 2 were composed of patients transplanted before and after November 2007, respectively, when plasma EBV DNA levels were periodically measured. *Results*: The study included 166 children, 89 (53.6%) boys, with a mean age of 12.2 ± 3.8 years. Prior to transplantation, 144 patients (86.7%) were EBV seropositive. Within a median follow-up period of 36 months, 11 of 22 seronegative children (50%) developed primary EBV infection. EBV reactivation was observed in 23 of 144 children (15.9%). Two patients with primary infection developed post-transplant lymphoproliferative disorder, one of whom died. Elevated serum creatinine levels or graft loss were not observed in any patient with EBV reactivation. *Conclusions*: EBV DNA monitoring by PCR in high-risk pediatric renal transplant recipients will provide early diagnosis and treatment of EBV infections.

Keywords

Children, Epstein–Barr virus infection, renal transplantation

History

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Introduction

Epstein–Barr virus (EBV), a member of the gamma herpes family, is a cause of infection in humans. In primary infection, the virus infects B lymphocytes; most of the proliferating B cells in the blood are removed with the host immune response. In immunosuppressed patients, EBV infected cells may proliferate and result in clinical problems ranging from asymptomatic seroconversion to high grade malignant lymphoma.[1–3]

Post-transplant lymphoproliferative disorder (PTLD) is one of the most significant complications of solid organ transplantation. PTLD incidence in pediatric renal transplant recipients varies between 1.2% and 7.1% in different series.[4–6] High mortality rates reaching up to 32–48% were reported in patients with PTLD.[5–7] Therefore, early recognition of recipients for high risk of PTLD via blood EBV DNA monitoring has gained great significance recently. Previous researches revealed the relation between rapid increases in peripheral blood EBV load and the onset of PTLD in patients with both solid organ and bone marrow transplantation.[8–10] Therefore, assessment of EBV viral load has become a part of the follow-up processes in patients with transplantation. The incidence of EBV-related diseases relevant to immunosuppressive medications is high in EBV seronegative pediatric transplant recipients. Clinical guidelines recommend monitoring of EBV viral load especially in pediatric patients and in EBV seronegative patients with an allograft from a seropositive donor.[11]

There has been no consensus among authors for the therapy of post-transplant EBV infections and PTLD. Most physicians first reduce immunosuppression once EBV infection has been diagnosed.[12] The goal to reduce the immunosuppression is to find a dose that allows restoration of an immune response against the EBV infections and PTLD without causing transplant rejection. It is necessary to continue the close monitoring of patients for early detection of acute rejection during reduction of immunosuppression.[13]

The objectives of this study were: (i) to investigate the EBV seropositivity rates at the pre-transplant period, (ii) to evaluate the frequency, time of occurrence, management and outcome of EBV infections and related complications during the post-transplant period and (iii) to assess effect of primary EBV infection and EBV reactivation on graft function in pediatric renal transplant recipients.

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Material and methods

Pediatric renal allograft recipients transplanted between August 1994 and December 2011 at our hospital was evaluated retrospectively. EBV viral capsid antigen (VCA) and Cytomegalovirus (CMV) IgG and IgM serology of all patients and donors were studied before the transplantation. The patients were divided into two groups. Group 1 was composed of patients transplanted before November 2007, and Group 2 that after 2007. Before November 2007, post-transplant monitoring of EBV was not routinely performed. EBV serological tests were employed only in case of clinical suspicion of the infection like fever, diarrhea, and sore throat or in case of high serum transaminase or creatinine levels. After November 2007, all cases were periodically monitored by determination of plasma EBV-DNA, via real-time quantitative polymerase chain reaction (PCR); the interval was specified as biweekly during the initial post-transplant 2 months, monthly between the 2nd and 12th month, bimonthly during the 2nd year and tri-monthly after the 2nd year. After post-transplant 2 years, serological methods were also used. Plasma and urine BK virus (BKV) and plasma CMV were periodically monitored with the same interval by quantitative real time PCR. EBV, CMV and BKV were also studied at the episodes of graft dysfunction.

Detection of EBV VCA IgM in serum and/or EBV DNA in plasma was defined as primary infection in seronegative recipients and as reactivation in seropositive recipients, respectively. The outcome of primary EBV infection and reactivation was classified as subclinical infection (asymptomatic viremia), symptomatic infection (fever, leukopenia, and/or organ involvement), or PTLD. The diagnosis of PTLD was based on biopsy findings in tissue samples.

Induction therapy was composed of methylprednisolone (MP) 500 mg/m^2 given during surgery, followed by a dosage of 80 mg/m^2/day on the second post-transplant day, with tapering down to 5 mg/m^2/day at the end of 3 months. Additionally, antithymocyte globulin (ATG) was administered to the patients with a deceased donor or with a HLA mismatch >3. As a maintenance immunosuppressive therapy, a calcineurin inhibitor [cyclosporine (CsA) or tacrolimus], an anti-proliferative agent [mycophenolate mofetil (MMF)/mycophenolic acid (MYF)/azathioprine (AZA)] or sirolimus and prednisone were administered. The target plasma trough level of tacrolimus was 10–15 ng/mL for the first 3 months, 8–10 ng/mL between 3 and 6 months and 4–8 ng/mL afterwards, and for CsA was 300–350 ng/mL for the first 3 months, 250–350 ng/mL between 3 and 6 months, 150–250 ng/mL for 6–12 months, and 100–150 ng/mL thereafter. Target trough levels for sirolimus were 5–8 ng/mL.

As a prophylactic antiviral therapy for cytomegalovirus (CMV), acyclovir (250 mg/m^2/day) and valacyclovir (2×500 mg in patients with GFR <15 mL/min/1.73 m^2; 2×1000 mg for GFR 15–30 mL/min/1.73 m^2; 3×1000 mg for GFR >30 mL/min/1.73 m^2) was given before and after January 2007, respectively. In seronegative patients for CMV, valganciclovir was used. Duration of antiviral prophylaxis was 6 months for those who underwent transplantation before November 2007 and 12 months thereafter.

Immunosuppressive dose modification was performed when plasma EBV DNA positivity (>1000 copies/mL) was detected. Firstly, in every patient according to the immunosuppressive drug dosage/situation and the blood level of CNI, either MMF dose was reduced by 30–50% or tacrolimus levels were decreased for a target tacrolimus trough of 4–6 ng/mL. In patients without a decrease in EBV DNA level after this modification, MMF was stopped and tacrolimus/CsA dose was reduced again. Patients were closely followed for signs of PTLD such as fever, lymphadenopathy, hepatosplenomegaly and gastrointestinal bleeding. Patients were also monitored for acute rejection by regular serum creatinine during clinic visits. Antiviral agents were continued if the patient were already receiving, otherwise they were not started. CMV-immunoglobulin or rituximab were not used for the treatment of EBV infection. These patients were monitored by 1- to 4-week intervals via PCR according to their EBV copy levels until the EBV viral load became negative.

Glomerular filtration rate (GFR) was estimated using the Schwartz formula.[14]

Biopsy findings were taken as the bases for PTLD diagnosis. Biopsy specimens were evaluated by an experienced pathologist to characterize the involved cell with markers such as CD20.

Serology

Epstein–Barr virus VCA and CMV IgM and IgG antibodies were determined in sera by enzyme immunoassay method using commercial kits (Radim, Pomezia RM, Italy; Viroimmun, Oberursel, Germany; Novatech, Dietzenbach, Germany; Euroimmun, Lübeck, Germany; BioMerieux, Marcy l'Etoile, France and Siemens, Erlangen, Germany) according to manufacturers' instructions.

Determination of EBV DNA load in plasma

Viral DNA was extracted from plasma samples by automated extraction systems (Qiagen BioRobot EZ1, Hilden, Germany) and EBV DNA was investigated by commercial quantitative real time PCR kits (Artus, Germany; Qiagen Artus, Hilden, Germany) according to manufacturers' instructions. The analytical detection limit of the kit is consistently 3.8 copies/μL.

Statistical evaluation

Statistical analysis of clinical data between the two groups was consisted of chi-squared test and Fisher's exact test, unpaired *T*-tests for parametric data and Mann–Whitney *U* test analysis for non-parametric data. Analyses were performed with IBM SPSS Statistics 19© Copyright SPSS Inc. software and p value < 0.05 was considered statistically significant.

Results

A total of 166 patients, 89 (53.6%) boys with a mean age of 12.2 ± 3.8 years (range: 1.5–18 years), were included in the study. Donor source was living related in 139 (83.7%) of the patients. Median follow-up period was 36 months (range: 3–137 months). Groups 1 and 2 were composed of 45 and 121

Table 1. Characteristics of patients before transplantation.

	EBV seropositive *N* (%)	EBV seronegative *N* (%)	*p* Value
Patients	144 (86.7)	22 (13.3)	
Median age at tx, year	13.2 (1.5–18)	10.2 (2–18)	0.05
Male/female	80 (55.5)/64 (44.5)	9 (40.9)/13 (59.1)	0.19
Live/deceased donor	121 (84)/23 (16)	18 (81.8)/4 (18.2	0.79
HLA mismatches ≥4 (*n*, %)	34 (23.6)	5 (22)	0.54
Time on dialysis, median (month)	12 (range: 1–108)	54 (16–108)	0.01
Follow-up duration, median (month)	29 (range: 3–137)	32.5 (7–81)	0.59
CMV seronegativity	6 (4.1)	5 (22.7)	0.007
Induction therapy, MP/MP-ATG	108 (75)/36 (25)	16 (72.7)/6 (27.3)	–
Primary immunosuppression			–
Prednisone-MMF/MYF-CSA	33 (22.9)	4 (18.1)	–
Prednisone-MMF/MYF-Tac	109 (75.6)	15 (68.2)	–
Prednisone-Sirolimus-CSA	2 (1.4)	1 (4.5)	–
Prednisone-AZA-Tac	–	2 (9.2)	–

Note: Tx, transplantation; MP, methylprednisolone; ATG, antithymocyte globulin; MMF, mycophenolate mofetil; MYF, mycophenoic acid; CSA, cyclosporine; Tac, tacrolimus; AZA, azathioprine.

Table 2. Primary renal disease of the patients.

Primary renal disease	Total 166, *n* (%)
Reflux nephropathy	24 (14.5)
Nephronophthisis/autosomal recessive polycystic disease	22 (13.3)
PUV	20 (12.0)
Focal segmental glomerulosclerosis	18 (10.8)
Renal aplasia/hypoplasia/dysplasia	17 (10.2)
Chronic glomerulonephritis	15 (9.0)
Neurogenic bladder	8 (4.8)
Cystinosis	5 (3.0)
Bardet–Biedel syndrome	3 (1.8)
Congenital nephrotic syndrome	2 (1.2)
Primary hyperoxaluria	2 (1.2)
Atypical hemolytic uremic syndrome (HUS)	2 (1.2)
Other (Prune Belly syndrome, Bartter Syndrome, Denys Drash Syndrome, Fanconi Syndrome)	4 (2.3)
Unknown	24 (14.5)

patients, respectively. Before the transplantation period, 144 patients (86.7%) were EBV seropositive. Also 155 patients (93.4%) were CMV seropositive. EBV seronegative patients were younger than seropositive ones (median 10.2 vs. 13.2 years, $p = 0.05$). Only three donors were EBV seronegative whose recipients were EBV seropositive. The demographic and clinical data of the seropositive and seronegative patients, and the primary renal disease of all patients are shown in Tables 1 and 2.

Primary infection

In Group 1, 5 of 45 patients were EBV seronegative. Primary infection was developed in two of these, one of whom developed PTLD at the 23rd month of post-transplant. She presented with gastrointestinal bleeding. Physical examination was unremarkable except abdominal distention. On laparotomy, perforation of stomach and caecum was detected. Pathological examination of biopsy specimen was compatible with PTLD. PCR analysis for EBV was strongly positive (4.9×10^6 copies/mL). In this period, all immunosuppressive (except steroid) drugs were stopped; acyclovir and later intravenous immunoglobulin was initiated; but we did not apply specific chemotherapy or anti-CD20 antibody due to severe infection. She died with septicemia on the 22nd day of hospitalization. Three of the remaining four patients were still seronegative, whereas the other was considered as previously having subclinical infection because EBV IgG positivity was detected by ELISA performed during this study (Table 3).

In Group 2, 17 of 121 patients were EBV seronegative, 9 of whom developed primary infection. Seroconversions were detected by serological methods in five patients with negative PCR results, who were considered as previously having subclinical infection. Without any clinical and laboratory deterioration, three patients were diagnosed as subclinical infection by PCR and one developed PTLD. The patient with PTLD was admitted with a high fever, sore throat, lack of appetite at 9-month post-transplantation. EBV DNA level measured by PCR was 2.9×10^5 copies/mL. Abdominal ultrasonography yielded a hypoechoic nodular lesion in the left lobe of liver, multiple millimetric hypoechoic lesions in both the liver and spleen. A liver biopsy showed CD20 (+) diffuses large B cells compatible with PTLD. After chemotherapy, within 14 months' follow-up after remission, renal function was preserved; neither EBV viremia nor PTLD recurred. This patient has been reported previously.[15] Elevated serum transaminase or creatinine levels or graft loss were not observed in any patient, except two who had low GFR due to BKV nephropathy or recurrent urinary infection due to vesicoureteral reflux (Table 3). The median time to first viremia was 9 months (range: 2–36), with a median follow-up time of 29 months (range: 8–48).

None of the patients with primary EBV infection in Groups 1 and 2 developed symptomatic infection during the study period (Table 4).

EBV reactivation

Epstein–Barr virus reactivation was observed in 23 of 144 EBV seropositive children (15.9%) by PCR. First viremia occurred at a median of 23-month post-transplant (range: 2–59).

In Group 1, 13 of 40 patients developed EBV reactivation. The median EBV DNA copy level was 352 (100–4604) copies/mL. Subclinical infection was detected in 12 patients. One patient had symptomatic infection.

Table 3. Characteristics of patients with primary EBV infection.

Patient No/Sex	Age at Tx (Month)	Donor	Induction therapy	Primary* immunsup.	Number of acute rejection	Method	Presentation	First viremia (month)	EBV viral load at diagnosis	CNI levels*** ng/mL	Immunsup. intervention	Result/last GFR
1/F	66	Live	MP	Tac	–	PCR	PTLD	23	4.9×10^6	6.2	Stop all immunsup.	exitus
2/M	120	Live	MP	Tac	–	PCR	PTLD	9	2.9×10^5	11.6	Stop all immunsup. except steroids	88
3/M	48	Live	MP	CsA	–	PCR	subclinical	36	2.5×10^4	65/304	Decrease CsA dose	52
4/F**	162	Deceased	MP-ATG	Tac	–	PCR	subclinical	2	957	12.7	Decrease Tac dose	32
5/F	102	Live	MP	Tac	1	PCR	subclinical	2	4330	7.2	Decrease MMF dose	90
6/F	162	Live	MP	Tac	–	serological	subclinical	–	–	–	–	79
7/M	180	Live	MP	Tac	2	serological	subclinical	–	–	–	–	66
8/F	200	Live	MP	Tac	–	serological	subclinical	–	–	–	–	85
9/F	198	Live	MP	Sirolimus	–	serological	subclinical	–	–	–	–	82
10/F	72	Deceased	MP-ATG	Tac	–	serological	subclinical	–	–	–	–	92
11/M**	204	Live	MP	CsA	–	serological	subclinical	–	–	–	–	44

Note: GFR, glomerular filtration rate; F, female; M, male; PTLD, post-transplant lymphoproliferative disorder; tx, transplantation; MP, methylprednisolone; ATG, antithymocyte globulin; CSA, cyclosporine; Tac, tacrolimus.
*All patients received mycophenolate mofetil or mycophenoic acid except patient 9.
**Patients 4 and 11 with low GFR had also BK nephropathy and recurrent urinary infection due to vesicoureteral reflux, respectively.
***Tac-0 or CsA-0 and 2 h.

Table 4. EBV-related complications in Group 1 and 2 patients.

	Group 1 ($n = 45$, 27.1%)	Group 2 ($n = 121$, 72.9%)	p
EBV seropositivity (n, %)	40 (88.9)	104 (85.9)	0.001
Subclinical infection	12	9	0.01
Symptomatic infection	1	1	0.41
PTLD	–	–	
EBV seronegativity (n, %)	5 (11.1)	17 (14.1)	0.8
Subclinical infection	1 (20)	8 (47)	0.79
Symptomatic infection	–	–	
PTLD	1 (20)	1 (5.9)	0.44

Note: PTLD, post-transplant lymphoproliferative disorder.

In Group 2, EBV reactivation was observed in 10 of 104 patients. The median EBV DNA copy level was 666.5 (221–2841) copies/mL. Subclinical infection developed in nine patients. One patient was diagnosed as symptomatic infection. EBV-related complications were shown on Table 4.

Primary EBV infection and EBV reactivation rates were similar in patients receiving tacrolimus or cyclosporine ($p = 0.72$ and $p = 0.31$, respectively) and in patients receiving MP or MP + ATG as induction therapy ($p = 1$ and $p = 0.61$, respectively). The median EBV DNA copy level was 2.5×10^4 copies/mL in 5 patients with primary infection and 600 copies/mL in 23 patients with EBV reactivation ($p = 0.018$).

Median tacrolimus level were 5.3 ng/mL (4.3–6.3), median CsA C0 level was 67 ng/mL (49–78) and C2 level 292 ng/mL (132–536) during EBV reactivation. The doses of the immunosuppressive drugs were reduced in 5 of 23 patients with EBV DNA >1000 copies/mL. Tacrolimus dose was reduced in two patients and MMF dose was reduced in three patients. The number of positive EBV results during EBV viremia was median 2 (1–5) results in these patients; six patients had only one positive EBV DNA. Elevated serum creatinine, graft loss or PTLD did not develop in any patient with EBV reactivation. Simultaneously CMV viremia (374 copies/mL) was observed in only one patient. The data of patients with primary infection and reactivation were shown on Table 5.

Epstein–Barr virus re-reactivation was observed in 10 of 23 children with previously EBV reactivation at a median of 38-month post-transplant (range: 11–96). The median EBV DNA copy level was 383 (78–1376) copies/mL.

In addition, graft and patient survival rates were analyzed: graft loss was in 14 (8.4%) patients, exitus was in 3 (1.8%) patients, transition with functioning graft to adult clinics was in 15 (9%) patients, lost of follow-up was in 7 (4.2%). The causes of graft loss were BK nephropathy in one patients, acute humoral rejection in six patients, recurrence of primary renal disease in two patients, and chronic allograft nephropathy in five patients. The causes of exitus were sepsis, PTLD and traffic accident in one patient each.

Discussion

In the present study, only 22 (13.3%) of 166 patients were EBV seronegative. This value is lower than that of North America and Europe, where it was reported as 19–57%.[16–19] In a study carried out in Turkey, 96.3% of 0–9 years of aged

Table 5. Characteristics of patients with primary EBV infection and EBV reactivation.

	Primary EBV infection ($n = 11$, 50%)	EBV reactivation ($n = 23$, 15.9%)	p
Mean age at tx (year)	11.4 ± 4.8	10.8 ± 3.9	0.69
Male/female, *n* (%)	4/7 (36.4/63.6)	12/11 (52.2/47.8)	0.38
Live/deceased donor, *n* (%)	9/2 (81.8/18.2)	20/3 (87/13)	0.69
Median follow-up duration (month)	29 (8–48)	48 (24–97)	0.01
First viremia, median (month)	9 (2–36)	23 (2–59)	0.47
EBV viral load at diagnosis median (copies/mL) (*n*/*n*)	2.5×10^4 ($957–4.9 \times 10^6$) 5/11	600 (100–4604) 23/23	0.018
Viremia duration (median, month)	2.0 (1–3)	1.0 (1–7)	NA
Number of positive EBV results during EBV viremia	3 (range: 2–10)	2 (range: 1–5)	NA
Acute rejection, *n* (%)	2 (18.2)	7 (30.4)	NA
BK viremia, *n* (%)	2 (18.2)	2 (8.7)	NA
BK nephropathy, *n* (%)	1 (9.1)	0	NA
Recurrent urinary infection, *n* (%)	2 (18.2)	3 (13.0)	NA

Note: NA, not available because of low numbers.

children was found to be seropositive.[20] The age when the primary EBV infection develops varies based on the socioeconomic status of the population. Earlier EBV seropositivity occurs in developing countries as well as in individuals coming from lower socioeconomic conditions.

Primary EBV infection experienced during the post-transplantation period is the major risk factor for PTLD development in renal transplant recipients. Since pediatric patients are mostly seronegative before the transplantation, they have a high PTLD risk.[21] PTLD incidence in pediatric renal transplant recipients varies between 1.2% and 7.1%.[4–6] In our study, PTLD incidence was determined as 1.2%. We thought that low PTLD risk in the current study may be related to low EBV seronegativity rates. Cleper et al.[22] reported that EBV-seropositive patients are at risk for aggressive late-onset lethal PTLD. However, in the present study, PTLD was not developed in any patient with EBV seropositive.

Epstein–Barr virus viremia may be related to asymptomatic or non-specific infection-related symptoms. Previously, the diagnosis of EBV viremia was based on the presentation of seroconversion; today it is based on the amplification of viral DNA with PCR. However, PCR results may vary based on sample type, while generally there is a correlation between peripheral blood leukocytes and complete blood values, but not with PCR values from plasma.[23] Although some studies showed a better specificity for plasma PCR as compared to peripheral blood leukocytes and complete blood, sensitivity can be lower.[3,4,24] Although complete EBV PCR panel is superior to determine EBV viral load, a single utilization of plasma EBNA PCR is assumed to be the method yielding the best results by some authors.[24] Tsai et al.[25] reported EBV PCR positivity in peripheral blood leukocytes in only 7 of 15 adult patients with PTLD (39%). However, Toyoda et al.[26] and Green et al.[10] reported no PTLD development during the monitoring periods by peripheral blood PCR. We also did not determined PTLD in any patient with negative EBV DNA by plasma PCR. And also Ishiara et al.[27] showed that plasma EBV loads (over 250 copies/mL) estimated by r-PCR may be useful to distinguish PTLD from other EBV-associated diseases or asymptomatic viremia. These findings support reliability of plasma EBV DNA monitoring in transplant recipients. However, in our study, although periodically performed PCR did not detect any positivity in five seronegative patients, EBV IgG was found positive by ELISA. This may be due to short-term presence of low level titers of EBV DNA during the late transplantation period with relatively low dose of immunosuppressive medications. For this reason, we suggest to monitor also EBV IgM antibodies in high-risk patients. Another explanation of these results was that we used plasma sample for the monitoring of EBV. Some studies showed that plasma PCR monitoring has a better specificity compared to peripheral blood leukocytes and complete blood, but sensitivity can be lower.[3] Plasma EBV DNA load of patients with primary EBV infection was significantly higher than the patients with reactivation, supporting the relationship of EBV negativity with EBV-related PTLD development.

Epstein–Barr virus DNA monitoring may result to decrease EBV-related complications like PTLD by providing early diagnosis and intervention.[11] The patient with PTLD in Group 1 was diagnosed at a late period when complications were developed, and she was lost. Contrary to this, in the patient who developed PTLD in Group 2, primary infection was early diagnosed, complete clinical and virological healing was observed with early and appropriate interventions. Besides, with immunosuppressive dose modifications when EBV DNA levels were >1000 copies/mL, a decrease in the viral load was observed in 3 asymptomatic patients with primary infection and 23 with EBV reactivation.

A universally accepted approach does not exist for the therapy of post-transplant EBV infections. Reduction of the dose of immunosuppressives is a therapy modality. While ganciclovir and valganciclovir have antiviral impact against EBV, acyclovir is not effective.[23] However, antiviral therapy in entire pediatric patients with acute EBV seroconversion is controversial.[1,12,13,28] By reducing immunosuppressives, natural T-cell mediated immune response suppressing duplication of EBV-infected B-cells is improved. In this way, B-cell proliferation may again be taken under control. Smets et al.[29] reported the relationship of low anti EBV T-cell activity with high viral load and elevated PTLD development risk. In the present study, CNI dose was reduced in two patients and MMF dose was reduced in one patient during primary infection; whereas tacrolimus dose was reduced in two patients and MMF dose was reduced in three patients at reactivation. Additional antiviral therapy was not applied. Some authors suggest that quantification of EBV-specific

immunity (cytotoxic T cells) may help to assess the efficacy of reduction of immunosuppression therapy and to predict the risk of EBV-associated symptoms such as PTLD.[30,31] In this study, we could not evaluate EBV-specific immunity.

Risk factors for the development of PTLD include the intensity of immunosuppression, the use of potent anti-lymphocyte antibodies, and a negative EBV serology before transplantation.[32] Factors related with poor PTLD prognosis were the involvement of multiple sites, tumor monoclonality, central nervous system (CNS) involvement, and late-onset (>1 year after transplant) PTLD.[32] In this study both PTLD cases were EBV seronegative before transplantation; and severe PTLD case were observed at the post-transplant 23rd months. Because of the low PTLD case number, we could not evaluate the risk factors underlying the development of PTLD and poor prognostic factors in our study population.

Most of PTLD cases occur during the initial 6 months of post-transplantation period. PTLD incidence is 20 times higher during the first month of post-transplantation.[33] In the present study, primary infection detected by PCR was observed at a median of post-transplant 9th month. Following a median 32 months of follow-up period, 11 cases were still seronegative. The first PTLD case was diagnosed at 23rd month and the second at 9th month. This may be related to prophylactic antiviral therapy duration of the patients. Especially during the period after the year 2007, patients took valacyclovir prophylaxis for 12 months. The patients might experience primary infection later due to the prophylaxis period. The patients were also taking intense immunosuppressive therapy during the first year after the transplantation and then immunosuppressive dose was reduced. Since lower immunosuppressive doses are taken in subsequent periods, mortality may be lower. Prophylaxis might cause to experience primary infection during the late post-transplantation period in which relative immunosuppressive doses are lower and consequently reduce the complications and PTLD development risk. But we did not evaluate impact of antiviral treatment statistically because of low number of PTLD.

Kidney disease Improving Global Outcomes (KDIGO)[11] clinical practice guideline for the care of kidney transplant recipients suggest that monitoring high-risk (donor EBV seropositive/recipient seronegative) renal transplant recipients for EBV by nucleic acid testing: once in the first week after transplantation; then at least monthly for the first 3–6 months after transplantation; then every 3 months until the end of the first post-transplant year; and additionally after treatment for acute rejection. During 3 years' period after November 2007, we intensively monitored all cases periodically. Our results suggest that KDIGO recommendations were compatible especially for seropositive recipients at transplantation; intensive monitoring may not be necessary for this group.

In conclusion, EBV DNA monitoring by PCR in transplanted children will provide early diagnosis of EBV infection and allow performing immunosuppressive modifications before the onset of symptoms. In particular, children with abnormally or persistently elevated EBV DNA titers should be evaluated for further investigation including imaging for PTLD, and cessation or reduction of immunosuppressive therapy should be considered as the first line treatment. Additionally, prospective studies with larger study groups will allow us to better understand the effect of EBV reactivation on graft survey.

Declaration of interest

E.C. and all coauthors declare that there are no conflicts of interest, relationships, and affiliations relevant to this article.

References

1. Green M, Michaels MG. Epstein–Barr virus infection and posttransplant lymphoproliferative disorder. *Am J Transplant*. 2013;13(Suppl 3):41–54.
2. Ponticelli C. Herpes viruses and tumours in kidney transplant recipients. The role of immunosuppression. *Nephrol Dial Transplant*. 2011;26:1769–1775.
3. Gulley ML, Tang W. Using Epstein–Barr viral load assays to diagnose, monitor, and prevent posttransplant lymphoproliferative disorder. *Clin Microbiol Rev*. 2010;23:350–366.
4. Morgans AK, Reshef R, Tsai DE. Posttransplant lymphoproliferative disorder following kidney transplant. *Am J Kidney Dis*. 2010; 55:168–180.
5. Holemes RD, Sokol RJ. Epstein–Barr virus and posttransplant lymphoproliferative disease. *Pediatr Nephrol*. 2002;16:456–464.
6. Srivastava T, Zwick DL, Rothberg PG, Warady BA. Posttransplant lymphoproliferative disease in pediatric renal transplantation. *Pediatr Nephrol*. 1999;13:748–754.
7. Allen U, Herbert D, Moore D, et al. Epstein–Barr virus-related post-transplant lymphoproliferative disease in solid organ transplant recipients, 1988–97: a Canadian multicentre experience. *Pediatr Transplant*. 2001;5:198–203.
8. Hoshino Y, Kimura K, Kuzushima K, et al. Early intervention in post-transplant lymphoproliferative disorders based on Epstein–Barr viral load. *Bone Marrow Transpl*. 2000;26:199–201.
9. Lucas KG, Burton RL, Zimmerman SE, et al. Semiquantitative Epstein–Barr virus (EBV) polymerase chain reaction for the determination of patients at risk for EBV-induced lymphoproliferative disease after stem cell transplantation. *Blood*. 1998;91: 3654–3661.
10. Green M, Cacciarelli TV, Mazariegos GV, et al. Serial measurement of Epstein–Barr viral load in peripheral blood in pediatric liver transplant recipients during treatment for posttransplant lymphoproliferative disease. *Transplantation*. 1998;66:1641–1644.
11. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant*. 2009;9(Suppl 3):S1–S155.
12. Frey NV, Tsai DE. The management of posttransplant lymphoproliferative disorder. *Med Oncol*. 2007;24:125–136.
13. Dharnidharka VR, Araya CE. Post-transplant lymphoproliferative disease. *Pediatr Nephrol*. 2009;24:731–736.
14. Schwartz GJ, Munõz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20: 629–637.
15. Koyun M, Hazar V, Akkaya B, et al. A case report: hepatic posttransplant lymphoproliferative disorder in a non-liver transplant patient. *Transplant Proc*. 2011;43:2102–2106.
16. Ellis D, Jaffe R, Green M, et al. Epstein–Barr virus-related disorders in children undergoing renal transplantation with tacrolimus-based immunosuppresion. *Transplantation*. 1999;68: 997–1003.
17. Shroff R, Trompeter R, Cubitt D, Thaker U, Rees L. Epstein–Barr virus monitoring in paediatric renal transplant recipients. *Pediatr Nephrol*. 2002;17:770–775.
18. Walker RC, Marshall WF, Strickler JC, et al. Pretransplantation assessment of the risk of lymphoproliferative disorder. *Clin Infect Dis*. 1995;20:1346–1353.
19. Suzuki T, Ikezumi Y, Okubo S, et al. Epstein–Barr virus DNA load and seroconversion in pediatric renal transplantation with tacrolimus immunosuppression. *Pediatr Transplant*. 2007;11:749–754.
20. Ozkan A, Kilic SS, Kalkan A, et al. Seropositivity of Epstein–Barr virus in Eastern Anatolian Region of Turkey. *Asian Pac J Allergy Immunol*. 2003;21:49–53.

21. Opelz G, Daniel V, Naujokat C, Döhler B. Epidemiology of pretransplant EBV and CMV serostatus in relation to posttransplant non-Hodgkin lymphoma. *Transplantation*. 2009;88:962–967.
22. Cleper R, Ben Shalom E, Landau D, et al. Post-transplantation lymphoproliferative disorder in pediatric kidney-transplant recipients – a national study. *Pediatr Transplant*. 2012;16:619–626.
23. Dharnidharka VR, Araya CE. Complications of renal transplantation. In: Avner ED, Harmon WE, Niaudet P, Yoshikawa N, eds. *Pediatric Nephrology*. 6th ed. Berlin Heidelberg: Springer-Verlag; 2009:1919–1939.
24. Tsai DE, Douglas L, Andreadis C, et al. EBV PCR in the diagnosis and monitoring of posttransplant lymphoproliferative disorder: results of a two-arm prospective trial. *Am J Transplant*. 2008;8: 1016–1024.
25. Tsai DE, Nearey M, Hardy CL, et al. Use of EBV PCR for the diagnosis and monitoring of post-transplant lymphoproliferative disorder in adult solid organ transplant patients. *Am J Transplant*. 2002;2:946–954.
26. Toyoda M, Moudgil A, Warady BA, Puliyanda DP, Jordan SC. Clinical significance of peripheral blood Epstein–Barr viral load monitoring using polymerase chain reaction in renal transplant recipients. *Pediatr Transplant*. 2008;12:778–784.
27. Ishihara M, Tanaka E, Sato T, et al. Epstein–Barr virus load for early detection of lymphoproliferative disorder in pediatric renal transplant recipients. *Clin Nephrol*. 2011;76:40–48.
28. Lee TC, Savoldo B, Rooney CM, et al. Quantitative EBV viral loads and immunosuppression alterations can decrease PTLD incidence in pediatric liver transplant recipients. *Am J Transplant*. 2005;5:2222–2228.
29. Smets F, Latinne D, Bazin H, et al. Ratio between Epstein–Barr viral load and anti-Epstein–Barr virus specific T-cell response as a predictive marker of posttransplant lymphoproliferative disease. *Transplantation*. 2002;73:1603–1610.
30. Sato T, Fujieda M, Maeda A, et al. Monitoring of Epstein–Barr virus load and killer T cells in pediatric renal transplant recipients. *Clin Nephrol*. 2008;70:393–403.
31. Guppy AE, Rawlings E, Madrigal JA, Amlot PL, Barber LD. A quantitative assay for Epstein–Barr Virus-specific immunity shows interferon-gamma producing CD8+ T cells increase during immunosuppression reduction to treat posttransplant lymphoproliferative disease. *Transplantation*. 2007;84: 1534–1539.
32. Knight JS, Tsodikov A, Cibrik DM, Ross CW, Kaminski MS, Blayney DW. Lymphoma after solid organ transplantation: risk, response to therapy, and survival at a transplantation center. *J Clin Oncol*. 2009;27:3354–3362.
33. Caillard S, Dharnidharka V, Agodoa L, Bohen E, Abbott K. Posttransplant lymphoproliferative disorders after renal transplantation in the United States in era of modern immunosuppression. *Transplantation*. 2005;80:1233–1243.