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Pediatric renal transplantation: A retrospective single-center study on epidemiology and morbidity due to EBV

A. Laurent[1] | A. Klich[2,3,4] | P. Roy[2,3,4] | B. Lina[5,6] | B. Kassai[3,7] | J. Bacchetta[1,4,5] | P. Cochat[1,3]

[1]Hospices Civils de Lyon, Service de Néphrologie, Rhumatologie et Dermatologie pédiatriques, Hôpital Femme-Mère-Enfant, Hospices Civils de Lyon, Lyon, France

[2]Hospices Civils de Lyon, Service de Biostatistique et de Bioinformatique, Lyon, France

[3]Université de Lyon, Lyon, France

[4]CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, Equipe Biostatistique-Santé, Villeurbanne, France

[5]Laboratoire de Virologie, Institut des Agents Infectieux IAI, Hospices Civils de Lyon, Hopital de la Croix Rousse, Lyon, France

[6]CIRI, Centre International de Recherche en Infectiologie, Virpath, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Université de Lyon, Lyon, France

[7]Centre d'Investigation Clinique de Lyon, 1407 Inserm-Hospices Civils de Lyon, Groupement Hospitalier Est, Hôpital Femme-Mère-Enfant, EPICIME, Bron, France

Correspondence
Audrey Laurent, Service de Néphrologie, Rhumatologie et Dermatologie pédiatriques, Hôpital Femme-Mère-Enfant, Bron, France.
Email: laurentaudr@gmail.com

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Abstract

Pediatric R-Tx patients are at high risk of developing EBV primary infection. Although high DNA replication is a risk factor for PTLD, some patients develop PTLD with low viral load. In this retrospective single-center study including all pediatric patients having received R-Tx (2003-2012 period), we aimed to identify risk factors for uncontrolled reactions to EBV (defined as the presence of a viral load >10 000 copies/mL or PTLD). A Cox proportional hazard model was performed. A total of 117 patients underwent R-Tx at a mean age of 9.7 ± 5.3 years, 46 of them being seronegative for EBV at the time of R-Tx. During follow-up, 54 patients displayed positive EBV viral load, 22 of whom presenting with primary infection. An uncontrolled reaction to EBV was observed in 24 patients, whilst 4 patients developed PTLD. Univariate and multivariate analyses suggested the following risk factors for an uncontrolled reaction: age below 5 years, graft from a deceased donor, ≥5 HLA mismatches, EBV-seronegative status at the time of R-Tx, and a secondary post-Tx loss of anti-EBNA. Monitoring anti-EBNA after R-Tx may contribute to the early identification of patients at risk for uncontrolled reaction.

KEYWORDS
EBV, EBV nuclear antigen, pediatric renal transplantation, PLTD, risk factors

1 | INTRODUCTION

EBV is a human virus from the *Herpesviridae* family, inducing a latent infection of B lymphocytes potentially controlled by EBV-specific cytotoxic T lymphocytes.[1-3] Patients who receive a R-Tx at a pediatric age are at risk of contracting EBV primary infection because of their frequent EBV-seronegative status at the time of Tx. Consequently, more frequently than others, these children develop EBV-associated complications,[4] and notably the severe PTLD.[5-7] PTLD occurs in 1.2-7.1% of graft recipients[8] and can be responsible for graft loss and even death.[9]

In 2012, the WHO revised the classification of PTLD. Indeed, they consider PTLD histopathology independently of time of onset since transplantation.[10,11] They separate disease into 1/plasma cell hyperplasia, which corresponds to early PTLD with exuberant lymphoid proliferation and preservation of normal tissue architecture despite the presence

Abbreviations: anti-EBNA, EBV nuclear antigen antibodies; anti-VCA, viral capsid antigen antibodies; AUC, area under the curve; CI, confidence intervals; CMV, cytomegalovirus; CS, corticosteroids; D+, positive donor; D−, negative donor; EBPG, European Best Practice Guidelines; EBV, Epstein-Barr virus; HR, hazard ratio; LMP, latent membrane protein; MMF, mycophenolate mofetil; PCR, polymerase chain reaction; PTLD, post-transplant lymphoproliferative disorder; R-Tx, renal transplant; R+, positive recipient; R−, negative recipient; WHO, World Health Organization.

of a mass effect (such as enlarged lymph nodes, tonsils, adenoid hypertrophy),[12,13] 2/non-early PTLD with polymorphic PTLD (polyclonal or monoclonal), and monomorphic PTLD, in which the normal architecture of the involved tissue is partially or completely destroyed.

To date, the three main risk factors linked to PTLD in pediatric graft recipients are EBV-seronegative status at the time of R-Tx, use of immunosuppressive therapies, and presence of a concomitant CMV primary infection.[6,14-16] High viral DNA replication (also known as increased viral load) has been recognized as a risk factor for developing PTLD.[17] Its detection in transplant patients by PCR helps physicians to trigger early diagnosis and management.[18,19] However, although the majority of patients with PTLD display increased EBV viral loads, some patients develop PTLD with low or even undetectable viral load. Conversely, some transplant recipients without PTLD display increased EBV viral load.[1,20]

Despite the link between the occurrence of EBV infection in pediatric patients having undergone R-Tx and the risk of developing PTLD, there is yet no consensus on viral load monitoring or the benefits of specific EBV chemoprophylaxis/treatment in this specific population. It is therefore interesting to understand the relationship between EBV seroconversion, the changes in EBV viral load, and the occurrence of PTLD after R-Tx.[5,21] The aim of this study was therefore to identify the risk factors for EBV-associated complications and presentation uncontrolled reaction to EBV after pediatric R-Tx.

2 | PATIENTS AND METHODS

2.1 | Patients and data collection

We performed a retrospective single-center study including all pediatric patients having received a R-Tx between January 2003 and December 2012 at our institution (Hôpital Edouard-Herriot before 2007, moving to Hôpital Femme Mère Enfant, Lyon, France). The inclusion criterion was to have received R-Tx before 18 years of age, whatever the rank (first or second R-Tx), the type of graft (deceased or living donor), or the type of Tx (isolated R-Tx, combined liver-kidney Tx CLKT, or combined heart-kidney Tx). During follow-up, a uncontrolled reaction to EBV was defined as the presence of a viral load >10 000 copies/mL or an onset of a histologically proven PTLD.

Clinical and biological data were extracted from the patient's medical charts by AL, and also from Cristal, a digital database for health professionals involved in organ transplantation in France (Agence de la Biomédecine). The study was approved by the local ethical committee (*Comité de Protection des Personnes Lyon Sud Est II*, IRB 00009118, session 7/10/2015, reference CAL-2015-52).

2.2 | Immunosuppressive therapies

Immunosuppressive treatments were administered according to a single standard local protocol. Induction therapy consisted of interleukin-2 receptor antagonist (2 doses, the first one given on the day of R-Tx, and the second one given 96 hours later), except in case of second R-Tx or R-Tx in immunized children (anti-thymocyte therapies). As early as 2006 and corresponding to the TWIST protocol,[22] corticosteroids were used for only 4 days in association with tacrolimus in children with low risk of EBV infection, especially in those without EBV mismatch (recipient seronegativity with donor EBV seropositivity) between donor and recipient at the time of R-Tx. However, when corticosteroids were used in association with cyclosporine A, they were used over longer periods with a gradual reduction scheme without withdrawal.

Calcineurin inhibitors and MMF were monitored through blood assays. During the first 3 months post-transplant, the therapeutic through target levels for cyclosporine A was 150-200 mg/L; the target levels were rather 100-150 mg/L thereafter. Before 2010, the time limit between these two different targets was 6 months instead of 3 months. For tacrolimus trough levels, the target was 5-10 mg/L regardless of the post-transplant delay before 2010. However, after 2010, the target was modified, from 10 to 15 mg/L during the 21 days post-transplant, to 5-10 mg/L between 21 days and 3 months, and eventually to 4-8 mg/L thereafter, so as to decrease nephrotoxicity and following the results of the TWIST study.[22] Starting from 2009, the target for the MMF AUC, as measured with a trough level, and then 20 minutes, 2 hours, and 3 hours after MMF intake, was 40-60 mg.h/L.[23]

The intensity of immunosuppression with MMF-AUC was assessed at least yearly, and at each visit for calcineurin inhibitors. Over-immunosuppression was defined as >1.5 mg/kg/d for azathioprine, >1200 mg/m²/d for MMF, >0.25 mg/kg/d for tacrolimus, or >10 mg/kg/d for cyclosporine A. The cumulative dose of corticosteroids was calculated over the course of the study period.

2.3 | Antiviral chemoprophylaxis

Starting from 2007, an anti-CMV chemoprophylaxis was used. Patients with high risk of primary CMV infection (ie, seronegative recipients with seropositive donors) were given ganciclovir followed by valganciclovir for 3 months and, starting from September 2012, valganciclovir was administered orally for 6 months. When the PCR became positive for CMV, the management was adapted according to changes in PCR results.

Starting from September 2012, chemoprophylaxis was also given to patients with a high risk of EBV reaction (ie, in patients seronegative for the anti-EBNA, but seropositive donors for EBV).

2.4 | EBV infection follow-up

The EBV viral load was measured on whole blood by real-time PCR and expressed in copies/mL using an in-house assay between 2003 and 2008, the EBVR-gene kit between 2008 and 2012 (Argene, Varhilles, France). In graft recipients seronegative for EBV at the time of R-Tx, a primary EBV infection was defined as a viral load >500 copies/mL. In graft recipients already seropositive for EBV at the time of R-Tx, a reactivation of EBV infection was defined as the increase in the viral load >500 copies/mL.

Serological surveillance was carried out with anti-EBNA and anti-VCA measured on plasma with a chemiluminescent microparticle

immunoassay on the Abbott ARCHITECT analyzer and before 2010 by the Siemens analyzer.

2.5 | Statistical analysis

Quantitative variables were summarized by means and standard deviations and qualitative variables by absolute and relative frequencies. The time to uncontrolled reaction to EBV was estimated by Kaplan-Meier method. Kaplan-Meier curves were generated to show differences between various patient subgroups.[24]

The analysis of the risk factors for uncontrolled reaction considered the following variables defined a priori: gender, age (under vs over 5 years old), the type of graft (deceased vs live donor), the type of Tx (isolated R-Tx vs combined Tx), the number of mismatches, the use of the immunosuppression before transplantation for immunologic disease (nephrotic syndrome, vascularitis, systemic erythematosus lupus; yes/no), and the viral status (CMV status of the donor, CMV status of the recipient, EBV status of the donor, EBV status of the recipient), EBV seronegativity with presence of anti-EBNA, the use of prophylaxis, the use of anti-CMV treatment, the monitoring of anti-EBNA after transplantation, the cumulative dose of corticosteroids, and over-immunosuppression.

A univariate survival analysis was performed using Cox regression[25,26] to check for differences between the various subgroups. This analysis provided hazard ratios with 95% CI. The independent variables found significant in this univariate analysis (as per the likelihood ratio test) were included in a multivariate Cox regression. The monitoring of anti-EBNA, the cumulated dose of CS, the cumulative dose of corticosteroids, and the overdose of immunosuppression considered as time-dependent covariates in the Cox regression.

The statistical analyses used R software, version 2.12.1 (Free Software Foundation, http://www.r-project.org). The significance level was set at 10% in the univariate analyses and at 5% in the multivariate analysis. Descriptive results for quantitative variables are expressed as mean ± SD.

3 | RESULTS

3.1 | Participant characteristics at the time of R-Tx

The baseline characteristics of the 117 participants are detailed in Table 1. The sex ratio was 1. The etiologies of end-stage renal disease were the following: congenital abnormalities of the kidney and urinary tract (N = 47, 40.2%), glomerulopathies (N = 32, 27.3%), ciliopathies (N = 12, 10.3%), primary hyperoxaluria type 1 (N = 12, 10.3%), and miscellaneous (N = 14, 12.0%). Among the 96 (82.1%) patients who underwent dialysis before R-Tx, 42 (36.9%) were on hemodialysis only, 34 (29.1%) on peritoneal dialysis only, and 20 (17.1%) patients underwent both dialysis techniques either alternately or simultaneously.

Fifteen patients received combined transplantations: 13 liver-kidney transplants and two heart-kidney transplants. Seven patients received a second R-Tx. The induction consisted of interleukin-2 receptor antagonist (basiliximab or daclizumab) in 109 (93.2%) patients, whereas only eight patients received polyclonal anti-thymocyte antibodies (thymoglobulin).

3.2 | EBV evolution

Twenty-four patients (20.5%) presented with uncontrolled reactions to EBV, of whom 17 (70.8%) were seronegative at the time of R-Tx. In 22 of these 24 patients, viremia was >10 000 EBV copies/mL. This high viral load was associated with PTLD in two patients whereas two patients presented PTLD with viral load <10 000 copies/mL. The number of viral load assessments per patient and per year was 4.3 ± 3.9. Among all patients, 22 (18.8%) benefited from antiviral prophylaxis: 12 for CMV mismatch, four for EBV mismatch, and six for both EBV and CMV mismatches. Age at R-Tx was 5.4 ± 4.6 years in the group with uncontrolled reaction vs 10.8 ± 4.9 years in the group without uncontrolled reaction to EBV ($P < .001$).

During follow-up, 54 (46.2%) patients displayed EBV viral load >500 copies/mL, corresponding to a primary infection in 22 (40.7%) of them. Four patients developed PTLD; they were all seronegative for EBV at R-Tx but all of them had seropositive donors.

Among the 32 patients who presented with a reactivation (presence of anti-VCA at the time of transplantation), five did not develop anti-EBNA whilst three acquired them. Among the 22 patients who developed a primary infection, four acquired neither anti-EBNA nor anti-VCA, whilst two acquired only anti-VCA.

Four patients presented with a PTLD due to EBV. One patient presented with an early lesion PTLD with exuberant lymphoid proliferation and preservation of normal tissue in a context of a viral load >10 000 copies/mL. He received rituximab for 2 years. He was seronegative for EBV at the time of transplantation and he did not acquire anti-EBNA. He died of pneumonia 1 year after the last infusion of rituximab. The three other PTLDs were non-early PTLDs. The first one was an atypical large B-cell lymphoma due to EBV but without viral load >10 000 copies/mL. The viral load was assessed three times a year (in 2003!), and the PTLD was diagnosed 1 year after R-Tx. This patient did not acquire EBV antibodies. She died 18 months after transplantation from undetermined encephalitis. Another patient presented with a Hodgkin's lymphoma due to EBV, with a viral load at PTLD diagnosis below 10 000 copies/mL, in a context of positive VCA and EBNA antibodies acquired after R-Tx during a primo-infection. The last patient presented with a Burkitt lymphoma due to EBV 7 years after R-Tx, after a long period with a viral load >10 000 copies/mL and in a context of bad compliance to medical follow-up. She also acquired VCA and EBNA antibodies after primo-infection.

3.3 | Post-R-Tx outcomes: patient survival, graft survival, rejection, disease recurrence

Table 2 shows the participants' characteristics after R-Tx. The follow-up period was 3.6 ± 2.7 years; this period was more prolonged in the

TABLE 1 Participant characteristics at baseline and transplantation

Variable	Without uncontrolled reaction to EBV n = 93	With uncontrolled reaction to EBV n = 24	All participants n = 117
Gender			
Boys	49 (52.7)	10 (41.7)	59 (50.4)
Girls	44 (47.3)	14 (58.3)	58 (49.6)
Age			
>5 y	76 (81.7)	8 (33.3)	84 (71.8)
<5 y	17 (18.3)	16 (66.7)	33 (28.2)
Age at transplantation	10.8 ± 4.9	5.4 ± 4.6	9.7 ± 5.3
Graft donor			
Live	11 (11.8)	1 (4.2)	12 (10.3)
Deceased	82 (88.2)	23 (95.8)	105 (89.7)
Age of donor	17.1 ± 11.1	11.3 ± 7.1	15.9 ± 10.6
Number of transplants			
1	86 (92.5)	16 (66.7)	102 (87.2)
>1	7 (7.5)	8 (33.3)	15 (12.8)
Cold ischemia time (h)	13.3 ± 6.2	14.4 ± 6.3	13.5 (6.2)
Number of HLA mismatches	3.0 ± 1.0	3.7 ± 1.2	3.2 ± 1.1
Number of mismatched HLA alleles			
0, 1, or 2	25 (26.9)	5 (20.8)	30 (25.6)
3	41 (44.1)	6 (25.0)	47 (40.2)
4	21 (22.6)	5 (20.8)	26 (22.2)
5 or 6	6 (6.5)	8 (33.3)	14 (12.0)
Number of DR mismatches	0.9 ± 0.7	1.1 ± 0.7	0.9 ± 0.7
Dialysis pretransplant			
No	18 (19.4)	3 (12.5)	21 (17.9)
Yes	75 (80.6)	21 (87.5)	96 (82.1)
Pretransplant immunosuppression			
No	74 (79.6)	22 (91.7)	96 (82.1)
Yes	19 (20.4)	2 (8.3)	21 (17.9)
EBV			
R−/D+	19 (20.4)	14 (58.3)	33 (28.2)
R+/D−	9 (9.7)	1 (4.2)	10 (8.5)
R+/D+	55 (59.1)	6 (25.0)	61 (52.1)
R−/D−	10 (10.8)	3 (12.5)	13 (11.1)
anti-EBNA at transplantation			
Negative	36 (38.7)	20 (83.3)	56 (47.9)
Positive	57 (61.3)	4 (16.7)	61 (52.1)
CMV			
R−/D+	20 (21.5)	6 (25.0)	26 (22.2)
R+/D−	17 (18.3)	5 (20.8)	22 (18.8)
R+/D+	19 (20.4)	3 (12.5)	22 (18.8)
R−/D−	37 (39.8)	10 (41.7)	47 (40.2)
Prophylaxis against CMV/EBV			
No	77 (82.8)	18 (75.0)	95 (81.2)
Yes	16 (17.2)	6 (25.0)	22 (18.8)
Induction immunosuppression			
Cyclosporine A	50 (53.8)	13 (54.2)	63 (53.8)
Tacrolimus short time CS	26 (28.0)	1 (4.2)	27 (23.1)
Tacrolimus long time CS	17 (18.3)	10 (41.7)	27 (23.1)

Values are expressed as number (%) or average ± standard deviation.

group with uncontrolled reaction (5.2 ± 2.9 years) vs the group without uncontrolled reaction (3.2 ± 2.5 years; $P = .004$).

Twenty-two patients (18.8%) presented isolated acute cellular rejection episodes, six (5.1%) isolated acute humoral rejections, and six mixed rejections (5.1%). Acute humoral rejections were generally treated with polyvalent immunoglobulins (5 patients). The treatment associated polyvalent immunoglobulins and rituximab in five patients and rituximab plus plasmapheresis in two. Acute cellular rejections were treated with methylprednisolone pulse therapy in 20 patients and oral prednisone in six. One patient with cellular rejection received adjusted doses of immunosuppressive agents (and notably increased doses of calcineurin inhibitors). Another patient with repeated acute cellular rejections received thymoglobulin. Last, one patient with mixed rejections received polyvalent immunoglobulin only.

Among the CLKT sub-group, three patients presented an acute hepatic rejection which was treated with corticosteroids. Six patients displayed recurrence of steroid-resistant nephrotic syndrome, of whom four were treated with plasmapheresis plus cyclophosphamide and two also received additional rituximab. Four patients presented a chronic rejection of whom three were treated with methylprednisolone pulse therapy and one with polyvalent immunoglobulins and rituximab.

Glomerular filtration rate as evaluated by the 2009 revised Schwartz formula (eGFR) was 73 ± 29 mL/min per 1.73 m^2 at 6 months, 77 ± 26 mL/min per 1.73 m^2 at 1 year, and 77 ± 26 mL/min per 1.73 m^2 at the end of follow-up.[27] Twelve patients (10.2%) lost their renal graft and went back to dialysis. The graft loss was linked to a vascular problem in five cases, to repeated acute rejections in three cases, to recurrences of the original disease in two cases, to poor compliance with medical therapy in one case, and eventually to EBV infection in the last case (because of a required tapering of immunosuppressive therapies and further severe and uncontrolled rejection).

TABLE 2 Patient characteristics during post-transplant care

Variables	Without uncontrolled reaction to EBV n = 93	With uncontrolled reaction to EBV n = 24	All participants n = 117
Follow-up period	3.2 ± 2.5	5.2 ± 2.9	3.6 ± 2.7
Loss of anti-EBNA			
Gain or maintenance	64 (68.8)	11 (45.8)	75 (64.1)
No gain	20 (21.5)	7 (29.2)	27 (23.1)
Gain and loss	9 (9.7)	6 (25.0)	15 (12.8)
Acute cellular rejection			
No	72 (77.4)	17 (70.8)	89 (76.1)
Yes	21 (22.6)	7 (29.2)	28 (23.9)
Acute vascular rejection			
No	84 (90.3)	21 (87.5)	105 (89.7)
Yes	9 (9.7)	3 (12.5)	12 (10.3)
Plasma exchange			
No	87 (93.5)	23 (95.8)	110 (94.0)
Yes	6 (6.5)	1 (4.2)	7 (6.0)
Rituximab without EBV reaction			
No	87 (93.5)	23 (95.8)	110 (94.0)
Yes	6 (6.5)	1 (4.2)	7 (6.0)
CMV treatment			
No	77 (82.8)	21 (87.5)	98 (83.8)
Yes	16 (17.2)	3 (12.5)	19 (16.2)
Cyclosporine			
No	40 (43.0)	7 (29.2)	47 (40.2)
Yes	53 (57.0)	17 (70.8)	70 (59.8)
Tacrolimus			
No	13 (14.0)	8 (33.3)	21 (17.9)
Yes	80 (86.0)	16 (66.7)	96 (82.1)
Mycophenolate mofetil			
No	1 (1.1)	0 (0.0)	1 (0.9)
Yes	92 (98.9)	24 (100)	116 (99.1)
Azathioprine			
No	75 (80.6)	15 (62.5)	90 (76.9)
Yes	18 (19.4)	9 (37.5)	27 (23.1)
Corticosteroids			
No	18 (19.4)	0 (0.0)	18 (15.4)
Yes	75 (80.6)	24 (100)	99 (84.6)

Values are expressed as number (%) or average ± standard deviation.

Four patients (3.4%) died over the study period. The first patient died 18 months post-transplantation from undetermined encephalitis in a context of EBV-induced lymphoma. The second patient died 56 months post-transplantation from pneumonitis in a context of B-cell immunosuppression after rituximab therapy for EBV-induced PTLD. The third patient died immediately after heart-kidney transplantation from primary heart dysfunction. The fourth patient died after her second R-Tx (first CLKT 10 years before) from sudden unexplained heart failure, as already reported.[28]

3.4 | Overall probability of non-occurrence of uncontrolled reaction to EBV

The survival analysis was conducted on 115 patients as viral load for EBV in two patients was not founding medical files, one because of early death (day 5 after combined kidney/heart Tx) and another because of early referral to another foreign center (day 27 after R-Tx). The median time to occurrence of a uncontrolled reaction to EBV was 9 years. In the entire cohort, the probability of non-occurrence of

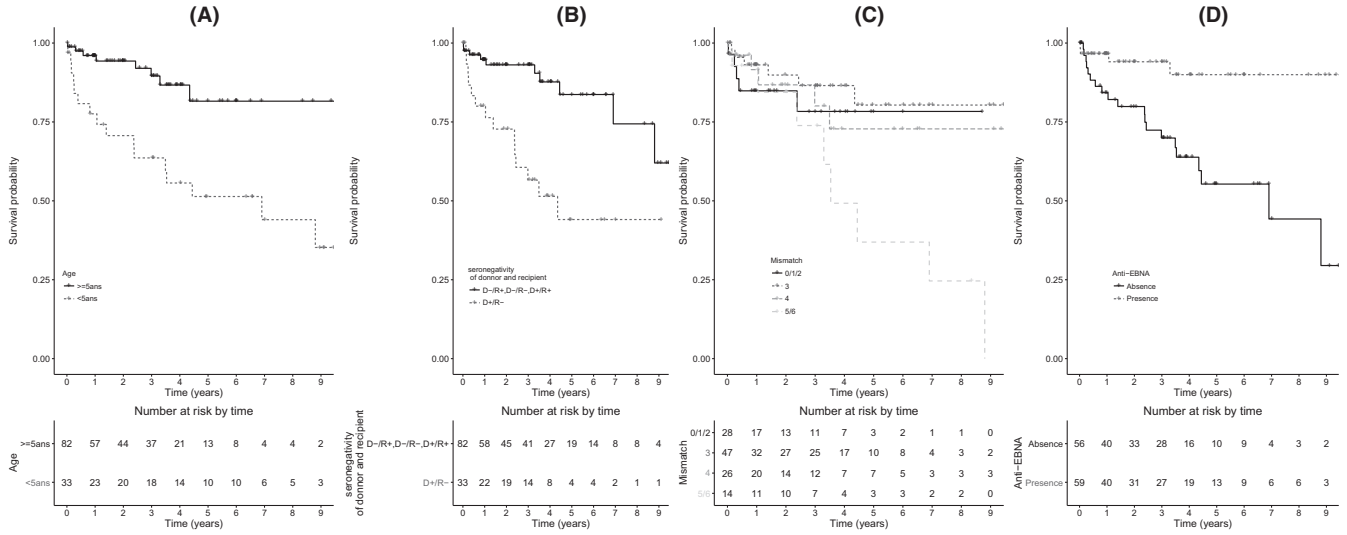

FIGURE 1 Probability of survival without uncontrolled reactions to EBV in transplant children according to age (A), seronegativity of donor and recipient (B), number of HLA mismatches (C), and presence of anti-EBNA at transplantation (D)

uncontrolled reaction to EBV at 1 and 9 years was estimated at 91% (95% CI: [85%; 96%]) and 55% (95% CI: [37%; 82%]), respectively. Figure 1 presents the probabilities of non-occurrence of uncontrolled reaction according to various participant characteristics.

3.5 | Risk factors for uncontrolled reaction to EBV

From univariate analysis, as summarized in Table 3, significant risk factors for uncontrolled reaction to EBV were the following: age below 5 years, deceased donor, combined Tx, more than 5 HLA mismatches, EBV mismatch at transplantation, and loss of anti-EBNA after R-Tx for patients whom were seropositives for anti-EBNA at the time of R-Tx or whom acquires anti-EBNA during the follow-up.

Children with combined transplantation were rather under 5 years of age; thus, variable was not introduced in the multivariable analysis. By multivariate analysis, as also summarized in Table 3, the factors that significantly increased the risk for uncontrolled reaction to EBV were the following: age below 5 years, deceased donor, more than 5 HLA mismatches, EBV mismatch at Tx, and loss of anti-EBNA after R-Tx.

4 | DISCUSSION

The main objective of the present study was to better understand EBV infection following pediatric R-Tx and to identify risk factors for uncontrolled EBV infections in this specific population. As such, the main findings of the present study are the following: 1/confirmation of previously described risk factors such a younger age at R-Tx and the presence of an EBV mismatch as risk factors for uncontrolled reaction to EBV, 2/description of two potential novel risk factors for uncontrolled reaction to EBV, namely an increased number of HLA mismatches, and the evolution of anti-EBNA during follow-up.

Among the 117 participants, four patients developed a PTLD. All four were seronegative for EBV at the time of R-Tx but had a seropositive donor for EBV. The previously reported prevalence of PTLD after pediatric R-Tx ranges between 1% and 10%[6,29–31] in pediatric R-Tx. In the present study, 3.4% of the participants developed PTLD in the entire cohort, but 8.7% of the seronegative patients at the time of Tx developed PTLD.

Among risk factors for uncontrolled reaction to EBV that were evaluated, two factors were identified by both univariate and multivariate analyses: recipient seronegativity with donor EBV seropositivity on one hand, and age below 5 years at the time of R-Tx on the other hand. The univariate analysis also identified "combined transplantation" as a risk factor for uncontrolled reaction to EBV; however, combined transplants in this cohort mainly correspond to CLKT in children with primary hyperoxaluria type 1, that are usually performed below 5 years of age, as previously reported by our group (18 CLKT between 1992 and 2013, median age of 3.6 years).[28] We considered it as a bias and did not take it into account in the multivariable model.

The survival analysis confirmed that seronegative recipients with seronegative donors were also at risk of uncontrolled reaction to EBV. Indeed, immediately after R-Tx, these recipients are at low risk of EBV infection: notably, their risk of both EBV reactivation and primary EBV infection from the graft is absent. However, during growth, most of these patients will encounter EBV and then become secondary at risk of severe EBV reaction due to the immunosuppressive therapies (P = .0002, log-rank test in Kaplan-Meier curve comparison).

The intensity of immunosuppression is a well-recognized risk factor for developing PTLD.[5,32] Indeed, the link between over-immunosuppression in young children and the risk of EBV infection has already been described.[31] In the present study, the use of immunosuppressive therapies before Tx did not appear to be a risk factor for uncontrolled reaction to EBV. Similarly, the cumulative corticosteroid dose or the presence of an over-immunosuppression did not seem to affect the risk of uncontrolled reaction to EBV, but we

TABLE 3 Risk factors for uncontrolled reactions to EBV in 117 transplanted children

	Univariate analysis		Multivariate analysis	
Variable	**HR [95% CI]**	***P*-value***	**HR [95% CI]**	***P*-value***
Sex (girls vs boys)	1.53 [0.67-3.45]	.31		
Age (<5 vs >5 y)	4.13 [1.74-9.78]	<.01	3.06 [1.03-9.09]	.04
Deceased (vs live) donor	4.02 [0.54-29.92]	.09	7.96 [0.85-73.81]	.02
Combined Tx (vs isolated)	3.69 [1.58-8.63]	.01		
≤2 HLA mismatches	1	.06	1	.02
3 HLA mismatches (vs ≤2)	0.58 [0.18-1.90]		0.65 [0.19-2.21]	
4 HLA mismatches (vs ≤2)	0.84 [0.24-2.93]		0.86 [0.22-3.36]	
≥5 HLA mismatches (vs ≤2)	2.49 [0.81-7.71]		4.45 [1.2-16.45]	
Pre-transplantation immune suppression (Yes vs No)	0.36 [0.09-1.55]	.12		
Donor CMV+ (vs donor CMV-)	0.64 [0.28-1.47]	.29		
Receiver CMV+ (vs receiver CMV-)	0.81 [0.35-1.90]	.63		
Receiver EBV-/Donor EBV +	4.1 [1.80-9.20]	<.01		
Receiver anti-EBNA +	1	<.01	1	<.01
Receiver anti-EBNA -/Donor EBV- (vs Receiver anti-EBNA +)	2.55 [0.57-11.45]		0.92 [0.14-5.91]	
Receiver anti-EBNA -/Donor EBV+ (vs Receiver anti-EBNA +)	6.47 [2.17-19.30]		8.21 [2.11-31.84]	
Administration of antiviral prophylaxis (Yes vs No)	1.80 [0.70-4.62]	.24		
Antiviral treatment for CMV (Yes vs No)	0.72 [0.22-2.44]	.59		
Cumulative dose of corticosteroids	1	.18		
Over-immunosuppression	0.86 [0.47-1.58]	.60		
Acquisition anti-EBNA	1	.009	1	.02
Loss of anti-EBNA (vs acquisition)	6.10 [2.41-17.34]		1.8 [0.56-5.76]	
No acquisition of anti-EBNA (vs acquisition)	1.44 [0.57-3.67]		0.32 [0.1-0.99]	

*Likelihood ratio test.

have to acknowledge that the retrospective design of the study is one major limitation of this kind of evaluation based on "cumulative drug-exposure." Besides we did not compare tacrolimus vs cyclosporine A in this manuscript because of a specific local policy in the choice of calcineurin inhibitors: indeed, in case of EBV mismatch, cyclosporine A is the calcineurin inhibitors of choice. In contrast, if the recipient is seropositive for EBV at the time of Tx, a TWIST protocol will be favored, with early corticosteroid withdrawal and double immunosuppressive regimen combining tacrolimus and MMF. As such, it is not surprising that tacrolimus appeared to be a protective factor against EBV uncontrolled reaction, but we did not consider this statistical significance relevant for clinical practice.

In the present study, displaying a CMV infection did not appear to be a risk factor for uncontrolled reaction to EBV, and this result can appear conflicting with the current published data.[6,33] Indeed, CMV disease can modify EBV replication through the modification of inflammatory cytokines such as tumor necrosis factor-α.[6] Moreover, CMV disease predicts the development of PTLD in primary EBV infection after liver Tx.[33] Jordan et al[34] also showed that CMV mismatch at Tx increases the risk of both opportunistic infection and PTLD. However, in the present cohort, neither the CMV status of the donor nor the one of the recipient affected the occurrence of uncontrolled reaction to EBV.

In 2002, the EBPG Expert Group on Adults Renal Transplantation recommended antiviral prophylaxis over 3 months in case of EBV-positive donor and EBV-negative recipient.[35] In that setting, some studies have also reported the benefits of valganciclovir as anti-CMV chemoprophylaxis in case of "CMV mismatch" in pediatric Tx. Valganciclovir also seems to be effective in reducing EBV viremia in liver transplant recipients.[36] In the present study, we did not find beneficial effects from prophylactic antiviral therapies, neither against CMV alone (since 2007) nor against CMV and EBV (since 2012). However, the small sample size and the changes in monitoring and care procedures that occurred over the course of the study do not allow us to draw any conclusion. A prospective trial designed to specifically assess the benefits of antiviral chemoprophylaxis in transplant children would be required to formally conclude, but it would be unethical to conduct it against placebo.

It is also important to discuss the role of the HLA system in the severity of EBV infection. Indeed, we found that the presence of ≥5 mismatches (vs ≤2; N = 14, and 30, respectively, in the entire cohort) more than doubled the risk of uncontrolled reaction to EBV. Caillard et al[15] also reported that >4 mismatches were a risk factor of PTLD. In our cohort, 5 mismatches or more were observed in 14 children, among them six CLKT. Also, nine of these 14 patients received their R-Tx during the first 4 years of follow-up and thus did not have a close monitoring of viral loads (2.5 ± 2.7 viral loads per patient per year in contrast to 4.3 ± 3.9 in the entire cohort).

Another important issue in the follow-up of pediatric Tx is the assessment of the risk of PTLD after Tx. Monitoring EBV viral loads is interesting to assess the risk of developing a severe EBV infection,[18,19] but the exact threshold still remains a matter of debate. Patients with great viral loads (≥10 000 copies/mL) are usually considered to be at higher risk of developing PTLD.[37,38] Based on the current literature on the topic, we recommend to control EBV viral load frequently, especially in case of EBV mismatch, namely with at least one control per week during the 2 first months after R-Tx, with a progressive lightening of follow-up.

However, in this cohort, we observed that the viral loads of two of four PTLD patients were always below 10 000 copies/mL. Indeed, the viral load was assessed 3 times a year in the first patient, who was diagnosed with PTLD 1 year after R-Tx. However, the second patient had only serologic follow-up after primo-infection because he acquires anti-EBNA, and viral load at PTLD diagnosis was <10 000 copies/mL. However, the literature confirms that PCR is not sensitive enough to be used alone.[37–39] Specifically, the different antibodies against EBV (and notably the anti-EBNA) should be followed regularly. Indeed, in latently infected asymptomatic individuals, B cells harboring EBV are usually in a resting state termed latency 0. B cells express EBNA1 in latency I, observed in Burkitt's lymphoma. In latency II (Hodgkin's disease and nasopharyngeal carcinoma), B cells express LMP1, LMP2, and EBNA1. During PTLD, B-cell proliferation expresses EBNA1 through EBNA 6, LMP1, LMP2A, and LMP2B in latency III.[40,41] The prognostic value of anti-EBNA could probably become interesting in the future, if other (and ideally prospective) studies confirm the present results: indeed, we found that, in addition to being EBV-negative at the time of R-Tx, the loss of anti-EBNA during the follow-up was also a significant risk factor for a uncontrolled reaction to EBV. Last, during conventional follow-up after Tx, other biomarkers such as LDH monitoring are probably of great interest, but still need to be assessed. In this series, LDH was difficult to interpret because of the change in biochemical assays and reference values during the study period.

This retrospective single-center study has obvious limitations and is subject to bias. The study probably lacked power, due to the relative limited number of patients. Moreover, even though we follow a strict local protocol (in terms of immunosuppression and drug target levels, clinical and biological follow-up, etc.), the frequency of viral load follow-up, the change in device to monitoring viral load, the policies of immunosuppression, and antiviral prophylaxis have changed over years in our center, as detailed in the methods. Even though statistical analyses took into account this particular point, this limitation should nevertheless be kept in mind. Two patients were excluded because they did not have any viral load for EBV in the medical files. Eventually, one of the main limits of this study is the need to choose an intermediate criterion for statistical analysis. We arbitrarily set the viremia threshold for uncontrolled EBV infection at 10 000 copies/mL whereas no specific evidence-based threshold or a threshold based on consensus exists, as already discussed above.[39] This is the main limit of this study because a viral load >10 000 copies/mL does not correspond to a PTLD.

In conclusion, within the field of uncontrolled reaction to EBV in pediatric renal transplantation, the present study was able to confirm the involvement of some previously described factors (age at transplantation, EBV D+/R− mismatch); however, it also suggests that monitoring anti-EBNA might help for a closer follow-up of children at risk of uncontrolled reaction to EBV. Multicenter prospective trials are required to determine an appropriate relevant EBV viral load threshold and to improve monitoring, prophylaxis, and management of EBV infection in pediatric transplant populations.

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CONFLICT OF INTEREST

The authors declare no financial or non-financial conflict of interests.

AUTHORS' CONTRIBUTIONS

AL: contributed to data collection, interpretation of results, and drafted the manuscript; AK and PR: contributed to data analysis and interpretation of results; BL: contributed to data analysis; BK: developed the study design; JB and PC: initiated the research and contributed to data analysis and interpretation of results; all authors contributed to critical revision of the manuscript and approved the final version.

ORCID

A. Laurent http://orcid.org/0000-0003-1282-4667

REFERENCES

1. Holmes MV, Caplin B, Atkinson C, et al. Prospective monitoring of Epstein-Barr virus DNA in adult renal transplant recipients during the early posttransplant period: role of mycophenolate mofetil. *Transplantation*. 2009;87:852-856.

2. Khanna R, Burrows SR. Role of cytotoxic T lymphocytes in Epstein-Barr virus-associated diseases. *Annu Rev Microbiol*. 2000;54:19-48.
3. Grywalska E, Markowicz J, Grabarczyk P, Pasiarski M, Rolinski J. Epstein-Barr virus-associated lymphoproliferative disorders. *Postepy Hig Med Dosw (Online)*. 2013;67:481-490.
4. Campe H, Jaeger G, Abou-Ajram C, et al. Serial detection of Epstein-Barr virus DNA in sera and peripheral blood leukocyte samples of pediatric renal allograft recipients with persistent mononucleosis-like symptoms defines patients at risk to develop post-transplant lymphoproliferative disease. *Pediatr Transplant*. 2003;7:46-52.
5. 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.
6. Shroff R, Rees L. The post-transplant lymphoproliferative disorder-a literature review. *Pediatr Nephrol*. 2004;19:369-377.
7. Taylor AL, Marcus R, Bradley JA. Post-transplant lymphoproliferative disorders (PTLD) after solid organ transplantation. *Crit Rev Oncol Hematol*. 2005;56:155-167.
8. Höcker B, Böhm S, Fickenscher H, et al. (Val-)Ganciclovir prophylaxis reduces Epstein-Barr virus primary infection in pediatric renal transplantation: (Val-)Ganciclovir reduces EBV primary infection. *Transpl Int*. 2012;25:723-731.
9. Maksten EF, Vase MØ, Kampmann J, et al. Post-transplant lymphoproliferative disorder following kidney transplantation: a population-based cohort study. *Transpl Int*. 2016;29:483-493.
10. Glotz D, Chapman JR, Dharnidharka VR, et al. The Seville expert workshop for progress in posttransplant lymphoproliferative disorders. *Transplantation*. 2012;94:784-793.
11. Mucha K, Foroncewicz B, Ziarkiewicz-Wróblewska B, Krawczyk M, Lerut J, Paczek L. Post-transplant lymphoproliferative disorder in view of the new WHO classification: a more rational approach to a protean disease? *Nephrol Dial Transplant*. 2010;25:2089-2098.
12. Colombini E, Guzzo I, Morolli F, et al. Viral load of EBV DNAemia is a predictor of EBV-related post-transplant lymphoproliferative disorders in pediatric renal transplant recipients. *Pediatr Nephrol*. 2017;32:1433-1442.
13. Parker A, Bowles K, Bradley JA, et al. Diagnosis of post-transplant lymphoproliferative disorder in solid organ transplant recipients - BCSH and BTS guidelines. *Br J Haematol*. 2010;149:675-692.
14. Dharnidharka VR, Sullivan EK, Stablein DM, Tejani AH, Harmon WE. Risk factors for posttransplant lymphoproliferative disorder (PTLD) in pediatric kidney transplantation: a report of the north american pediatric renal transplant cooperative study (NAPRTCS) 1. *Transplantation*. 2001;71:1065-1068.
15. 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.
16. Sampaio MS, Cho YW, Shah T, Bunnapradist S, Hutchinson IV. Impact of Epstein-Barr virus donor and recipient serostatus on the incidence of post-transplant lymphoproliferative disorder in kidney transplant recipients. *Nephrol Dial Transplant*. 2012;27:2971-2979.
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. Baudouin V, Dehée A, Pédron-Grossetete B, et al. Relationship between CD8+ T-cell phenotype and function, Epstein-Barr virus load, and clinical outcome in pediatric renal transplant recipients: a prospective study. *Transplantation*. 2004;77:1706-1713.
19. 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.
20. Tanaka E, Sato T, Ishihara M, et al. Asymptomatic high Epstein-Barr viral load carriage in pediatric renal transplant recipients: asymptomatic high EBV load in renal transplantation. *Pediatr Transplant*. 2011;15:306-313.
21. Riddler SA, Breinig MC, McKnight JL. Increased levels of circulating Epstein-Barr virus (EBV)-infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of posttransplant lymphoproliferative disease in solid-organ transplant recipients. *Blood*. 1994;84:972-984.
22. Zhao W, Elie V, Roussey G, et al. Population pharmacokinetics and pharmacogenetics of tacrolimus in de novo pediatric kidney transplant recipients. *Clin Pharmacol Ther*. 2009;86:609-618.
23. Zhao W, Fakhoury M, Deschênes G, et al. Population pharmacokinetics and pharmacogenetics of mycophenolic acid following administration of mycophenolate mofetil in de novo pediatric renal-transplant patients. *J Clin Pharmacol*. 2010;50:1280-1291.
24. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457.
25. Therneau TM, Grambsch PM. Modeling survival data: extending the Cox model. In: Dietz K, Gail M, Krickeberg K, Samet J, Tsiatis A, eds. *Statistics for Biology and Health*. New York, NY: Springer; 2000:1-287.
26. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep*. 1966;50:163-170.
27. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20:629-637.
28. Duclaux-Loras R, Bacchetta J, Berthiller J, et al. Pediatric combined liver–kidney transplantation: a single-center experience of 18 cases. *Pediatr Nephrol*. 2016;31:1517-1529.
29. Hocker B, Fickenscher H, Delecluse H-J, et al. Epidemiology and morbidity of Epstein-Barr virus infection in pediatric renal transplant recipients: a multicenter, prospective study. *Clin Infect Dis*. 2013;56:84-92.
30. Holmes RD, Sokol RJ. Epstein-Barr virus and post-transplant lymphoproliferative disease. *Pediatr Transplant*. 2002;6:456-464.
31. McDonald RA, Smith JM, Ho M, et al. Incidence of PTLD in pediatric renal transplant recipients receiving basiliximab, calcineurin inhibitor, sirolimus and steroids. *Am J Transplant*. 2008;8:984-989.
32. Sampaio MS, Cho YW, Shah T, Bunnapradist S, Hutchinson IV. Association of immunosuppressive maintenance regimens with posttransplant lymphoproliferative disorder in kidney transplant recipients. *Transplantation*. 2012;93:73-81.
33. Mañez R, Breinig MC, Linden P, et al. Posttransplant lymphoproliferative disease in primary Epstein-Barr virus infection after liver transplantation: the role of cytomegalovirus disease. *J Infect Dis*. 1997;176:1462-1467.
34. Jordan CL, Taber DJ, Kyle MO, et al. Incidence, risk factors, and outcomes of opportunistic infections in pediatric renal transplant recipients. *Pediatr Transplant*. 2016;20:44-48.
35. EBPG Expert Group on Renal Transplantation. European best practice guidelines for renal transplantation. Section IV: long-term management of the transplant recipient. IV.6.1. Cancer risk after renal transplantation. Post-transplant lymphoproliferative disease (PTLD): prevention and treatment. *Nephrol Dial Transplant*. 2002;17(Suppl 4):31-33, 35-36.
36. Hierro L, Díez-Dorado R, Díaz C, et al. Efficacy and safety of valganciclovir in liver-transplanted children infected with Epstein-Barr virus. *Liver Transpl*. 2008;14:1185-1193.
37. Sato T, Fujieda M, Tanaka E, et al. Monitoring of Epstein-Barr virus load and antibody in pediatric renal transplant patients. *Pediatr Int*. 2008;50:454-458.
38. 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.
39. Smith JM, Dharnidharka VR. Viral surveillance and subclinical viral infection in pediatric kidney transplantation. *Pediatr Nephrol*. 2015;30:741-748.

40. Hopwood P, Crawford D. The role of EBV in post-transplant malignancies: a review. *J Clin Pathol*. 2000;53:248-254.
41. Tierney RJ, Steven N, Young LS, Rickinson AB. Epstein-Barr virus latency in blood mononuclear cells: analysis of viral gene transcription during primary infection and in the carrier state. *J Virol*. 1994;68:7374-7385.

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