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Epidemiology and Outcome of Chronic High Epstein-Barr Viral Load Carriage in Pediatric Kidney Transplant Recipients

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Abstract

Introduction—The development of Epstein-Barr virus (EBV) infection and post-transplant lymphoproliferative disorder (PTLD) is normally associated with a high EBV viral load in peripheral blood. Observations have previously identified existence of a chronic EBV high load (CHL) carrier state which demonstrated variable outcomes based upon the organ which was transplanted. Data defining the incidence and outcome of CHL in pediatric kidney transplantation (KTx) are not well described.

Methods—The charts of children undergoing isolated KTx at Children's Hospital of Pittsburgh between January 2000 and December 2014 were retrospectively reviewed. EBV loads in the peripheral blood were routinely measured as part of surveillance protocols at our center. CHL was defined as the presence of high load for >50% of samples for ≥6 months. PTLD was defined histologically using WHO definitions.

Results—Of 188 isolated KTx recipients, we identified a total of 16 (8%) children who developed CHL carrier state. No patient developed EBV-driven late onset PTLD. Age at the time of KTx was significantly lower in CHL group (median 3.9 years, interquartile range: IQR 2.9-6.6, p=0.0004). Children in the CHL group were more likely to be EBV seronegative prior to KTx (94%, 15/16), compared to the undetectable viral load (UVL) and low viral load (LVL) groups (55% and 50%, respectively, p<0.002). The median duration of CHL carrier state was 20 months (IQR 10.7-35.8). Fifteen of the 16 CHL carriers experienced spontaneous resolution of CHL carrier state. Children in the CHL group were younger at the time of primary EBV infection (p=0.023). Finally, antiviral medication was not effective in either preventing or decreasing the EBV viral load in blood (p=0.84).

Conclusion—Overall incidence of late-onset PTLD is very low compared to heart and intestinal transplant, even though KTx recipients can develop CHL carrier state. The CHL carriers in KTx

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recipients were EBV seronegative prior to transplant and were younger both at the time of KTx and at the time of primary EBV infection compared to those in the UVL and HVL groups. Antivirals did not prevent EBV infection or decrease EBV viral loads.

Introduction

Epstein Barr virus (EBV) infections account for significant morbidity and mortality in children undergoing solid organ transplantation (SOT). EBV is associated with a range of clinical manifestations and syndromes including the majority of post-transplant lymphoproliferative disorders (PTLD) diagnosed in children (1). The measurement of EBV load by polymerase chain reaction (PCR) in the peripheral blood has been used for diagnosis of EBV infection allowing for preemptive intervention against EBV disease and PTLD (2). The use of serial monitoring of EBV loads has identified a population of children who develop and subsequently maintain very high viral loads in the absence of clinical symptoms over long periods of time, a condition previously termed the “Chronic EBV High Load (CHL) carrier state”(3–5). Prior investigations of the long-term clinical significance of the asymptomatic CHL carrier state amongst pediatric heart, liver and intestinal transplant (Tx) recipients identified different outcomes based on organ type. Children undergoing pediatric heart recipients with CHL had a 45% chance of developing **PTLD** (3), while CHL carriers amongst pediatric liver and intestinal Tx recipients had lower incidences (3% and 11%, respectively) of PTLD (4, 5). The incidence and outcome of the EBV CHL carrier state in pediatric kidney transplant (KTx) recipients has not been well described (6, 7). In addition, while many centers use antiviral medication to prevent EBV infection after transplant, its impact has not been fully studied (8, 9). The purposes of this current study are to review the incidence of EBV infection including CHL carrier state and PTLD and to investigate the impact of antiviral therapy on EBV infection in KTx recipients.

Material and Methods

A retrospective chart review of all 222 children undergoing KTx at the Children’s Hospital of Pittsburgh of UPMC (CHP) between January 2000 and December 2014 was performed. The electronic medical records and transplant database were reviewed and data was systematically extracted including age at KTx, gender, induction therapy, maintenance immunosuppression, antiviral medication use and pre-Tx EBV serology from both donors and recipients as available. Data from children undergoing re-transplantation were censored at the time of their second procedure. Longitudinal, serial measurements of EBV, BK virus (BKV) and cytomegalovirus (CMV) viral loads (as measured by either PCR or CMV antigenemia) were recorded.

Serial measurement of EBV load in the peripheral blood using quantitative EBV DNA PCR assays was introduced at CHP in the mid-1990s. EBV loads were initially determined using a quantitative competitive PCR performed on peripheral blood lymphocytes (PBMC) with results expressed as genome copies per 10^5 PBMC (10). Since October 2002, EBV load measurement has been performed on whole blood using a real-time PCR method with results expressed as genome copies per mL whole blood (2). A previously published comparisons of these two assays revealed a strong correlation between them, allowing inclusion of all the

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available data from both assays (2). Prior to 2002, EBV load monitoring was performed every month for the first 6 months followed by measurement every 3 months until 2-year post KTx. Since 2002, CHP standard viral surveillance in all pediatric KTx recipients consists of quarterly measurement of whole blood for EBV and CMV regardless of risk factors such as pre-Tx EBV/CMV serologies. For routine CMV monitoring, CMV antigenemia assay was used between 2002 and 2006, replaced by CMV PCR after 2006. In 2008, the protocol was modified to include increasing the frequency of surveillance to monthly after primary detection of EBV in recipients who were EBV seronegative prior to transplantation, when detectable loads continued to increase, or when the EBV load exceeded 16,000 genome copies/mL. Monthly surveillance is continued until the viral loads are stabilized, decreasing and/or maintained less than 16,000 genome copies/mL followed by regular quarterly surveillance. Additional loads were measured with clinical event such as a for-cause renal biopsy or other indication determined by physicians. In parallel, routine measurement of urine and plasma BKV loads was introduced in 2010. The study was approved by the University of Pittsburgh Institutional Review Board (PRO15010060).

Definitions

EBV load carrier states were defined as previously published (3–5, 11): Undetectable viral load (UVL) carriers had EBV viral loads that were undetected or detected but not quantifiable (less than 100 genome copies/mL) in more than 80% of all data points; CHL carriers had EBV loads exceeded 16,000 genomic copies/mL blood in more than 50% of load data points over a minimum of 6 months or longer. Chronic low viral load (LVL) carriers included those children not meeting criteria for either the UVL or CHL carrier state. The onset of the CHL carrier state was defined when the initial elevated load associated with the persistent period of high EBV load was measured; while resolution of CHL was defined as the initial date of EBV load less than 16,000 when subsequent EBV loads remain less than 16,000 genome copies/mL. The diagnosis of post-Tx primary EBV infection was made by the finding of a positive PCR result and/or seroconversion in a child who was EBV seronegative prior to transplant. BKV viremia was defined by the presence of at least one measurement of >1000 copies/mL plasma by DNA PCR, which is the threshold for quantification. Similarly, CMV antigenemia/viremia was defined by the presence of at least one measurement of either positive CMV antigenemia or positive CMV PCR in whole blood. EBV disease was defined when KTx recipients with EBV load developed symptoms such as prolonged fever, malaise, night sweat, lymphadenopathy and adenotonsillitis which were routinely assessed during each clinic visit. The diagnosis of BKV/CMV disease were made based on symptoms and/or pathology. PTLD was defined histologically using WHO definitions. PTLD was considered EBV driven by the presence of positive stain for EBV encoded small nuclear RNAs (EBER). Late onset PTLD was defined by PTLD occurring after the first post-Tx year.

Immunosuppressive and Antiviral Treatment Regimens

Standard immunosuppression evolved during the study period. Tacrolimus and mycophenolate mofetil were used throughout the study period. The first line induction therapy varied with early KTx recipients (2000-2004) receiving anti-thymoglobulin induction with methylprednisolone while those transplanted after 2004 received

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alemtuzumab with methylprednisolone. For children, whose EBV loads continued to climb or who newly met the criteria of CHL carrier state, initial consideration has been to decrease or discontinue mycophenolate mofetil; tacrolimus was decreased as necessary for those whose loads failed to fall in response to the reduction or discontinuation mycophenolate mofetil.

Antiviral prophylaxis with ganciclovir or valganciclovir was given for the first 6 months for children with pre-Tx positive EBV serologies and for the first 12 months for the patient with pre-Tx negative EBV serologies after KTx. Ganciclovir or valganciclovir were also preemptively given by clinicians when lymphocyte depleting re-induction therapy was being initiated due to rejection, in the presence of CMV antigenemia or viremia, rising EBV viral loads or the EBV CHL carrier state regardless of symptomatology in addition to the previously noted titration of immunosuppression.

Statistical Analysis

Each demographic data across groups was analyzed with Chi-square and One-way ANOVA (parametric values) or Kruskal-Wallis test (nonparametric values). Efficacy of antiviral therapy was analyzed in two ways: 1. Comparison of incident rate of primary EBV infection in pre-Tx EBV seronegative children between two segmented periods, and 2. Generalized linear model adjusted for dependent data to compare mean EBV viral loads amongst CHL carriers.

Results

A total of 222 children underwent KTx at CHP between 2000 and 2014. Twenty-six children undergoing multi-organ Tx and eight children who had only one or no EBV loads measurements were excluded, respectively. The remaining 188 children were eligible for this study and were included in the full analysis. The characteristics analyzed for the entire study cohort and by viral load carrier state (UVL, LVL and CHL) are shown in Table 1. The median age at the time of transplant for the entire cohort was 12.1 years with median follow-up of 6.9 years after KTx. Sixty percent of those children were male and 76% received induction therapy with alemtuzumab. One hundred and seven of the children were EBV negative; 93 of the EBV seronegative children received an organ from an EBV seropositive donor (D+/R-).

One hundred thirteen, 59, and 16 children met the definition of UVL, LVL and CHL, respectively (Table 1). Age at the time of KTx was significantly lower in CHL group (median 3.9 years, interquartile range: IQR 2.9-6.6, p=0.0004) compared to LVL and UVL groups, respectively. Children in the CHL group were more likely to be EBV seronegative prior to KTx (94%, 15/16), compared to the UVL and LVL groups (55%, and 50%, respectively, p<0.002). There were no differences between sex, follow-up duration or type of induction therapy between the three viral load carrier states. Of interest, there was no statistical difference between groups for the incidence of BKV viremia of BK nephropathy. The incidence of CMV antigenemia/viremia was lower in CHL group (p=0.052) while all the CHL children had negative CMV serology before Tx (p=0.012). There was no child in any group who developed CMV disease during the study period.

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Amongst the 16 CHL carriers, the median peak EBV load was 39,000 genome copies/mL (range 26,000-140,000). Fifteen of the 16 CHL carriers were EBV seronegative prior to transplant with 11 developing primary infection within one year after KTx. One child with positive EBV serologies at the time of KTx did not have an identifiable clinical event (e.g. rejection) preceding his CHL carrier state. Notably, he was on tacrolimus monotherapy with levels ranging between 8 and 12 ng/mL. His CHL persisted over 14 months before spontaneous resolution. The median duration of CHL carrier state for all was 20 months (IQR 10.7-35.8). Fifteen of the 16 CHL experienced spontaneous resolution of CHL carrier state. One patient received intravenous immunoglobulin who recovered from CHL belatedly and none received rituximab. Two of the 16 children experienced multiple episodes of CHL separated by 6 to 9 months of having loads serially measured below 16,000 genome copies/mL.

Only one patient developed symptomatic EBV disease classified as EBV enteritis (not PTLD) while being an active CHL carrier. This patient was, subsequently diagnosed with inflammatory bowel disease with unclear correlation with CHL carrier state. None of the other 15 CHL carriers experienced symptomatic EBV disease or PTLD while in CHL carrier state during the study period.

A total of 107 (57%) children had negative EBV serologies prior to transplant. Thirty percent (32/107) of EBV naïve children developed primary EBV infection within 1 year post-Tx and overall 52% (56/107) developed a primary EBV infection during the study. These 56 children were characterized into the 3 EBV load groups; there were 11 UVL, 30 LVL and 15 CHL respectively (Table 2). Children in the CHL group were younger at the time of primary EBV infection (p=0.023) and appeared younger at the time of KTx, though the latter was not statistically significant (p=0.12). Children categorized as UVL appeared to develop primary infection much later after transplant (median: 24.4 months) compared to those categorized as LVL or CHL (median 7.1 and 7.3 months, respectively), though this did not show statistical difference (p=0.81).

The efficacy of antiviral therapy for the prevention of EBV infection and for the CHL carrier state were also investigated. When the incidence of EBV infection (infection/patient/year) during the first 12 months after Tx (on antivirals) was compared to the incidence during the second 12 months (off antivirals) after transplant, it was higher during the first 12 months (p=0.0006). A linear regression model was applied to evaluate the impact of antiviral treatment with ganciclovir and/or valganciclovir on the EBV viral loads. There was no significant difference of viral loads between “on medication” vs “off medication” (p=0.84).

Discussion

PTLD is a life-threatening complication for children undergoing SOT (1, 7, 12, 13) including those undergoing KTx where the overall incidence of early and late-onset PTLD has been reported to range between 5-8%. Efforts to enhance the diagnosis and to improve outcome of EBV infection in SOT recipients has led to the routine use of EBV viral load monitoring as part of the clinical management of transplant recipients. In turn, this widespread use of EBV load monitoring has led to the recognition that many pediatric Tx

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recipients will maintain high EBV loads in the peripheral blood for prolonged periods of time, prompting investigation and characterization of different patterns of EBV load carriage states (3, 14). Initial observations in heart Tx recipients identified the CHL carrier state, even if asymptomatic, as a significant risk factor for developing PTLD (3). However, much lower rates of PTLD were found in children with CHL undergoing liver or intestinal Tx. The current study adds to the understanding of EBV load carrier states by addressing the incidence and outcome of the CHL carrier state among KTx recipients using the same definitions and EBV assay as the prior studies of CHL in pediatric heart, liver and intestinal Tx recipients.

The incidence of CHL carrier state and late onset PTLD in KTx recipients was the lowest across the different organ transplantation

The incidence of CHL in children undergoing KTx was 8% and no child developed late-onset PTLD in our study cohort, which represents the lowest incidence of both CHL and PTLD of all the different types of pediatric SOT recipients. However, it is worth noting that one patient who had been a CHL carrier developed late-onset PTLD, 29 months after KTx after the study closed. As Moudgil et al. reported incidence of late-onset PTLD as 2% (2/85) in asymptomatic persistent EB viral load, the very low incidence of late-onset PTLD was consistent with other previous studies. Similar to other studies, risk factors for CHL carrier state in the current study included younger age at the time of transplantation and being EBV seronegative prior to transplant (15, 16). Given the increasing seroprevalence of EBV by age in healthy individuals (17), these two are likely dependent factors although this was not proven in the current study by multivariate analysis due to small sample number. Interestingly, age at the time of primary EBV infection was also significantly younger in CHL groups while the timings of primary EBV infection after Tx was not different between groups. Given the inefficient development of effector T cell in younger age, it is plausible EBV infection in younger age by itself is an additional risk factors for CHL carrier state (18). The kinetics of BKV and CMV were analyzed by EBV viral load group with the assumption that the intensity of immunosuppression is a key factor promoting the development of uncontrolled chronic viral infection after KTx (19). However, there was no differences in the incidence of BK viremia or BK nephropathy amongst the groups. Interestingly, despite having the highest rate of CMV seronegative children prior to KTx, CMV antigenemia/viremia was not observed in CHL group. This may be explained, in part, by the frequent use of antiviral therapy in CHL group. Regardless, our data did not support the increased risk for other viral infection in CHL group.

When compared with previously published data on the outcome of CHL carrier state with other pediatric SOT recipients at CHP, there was statistically lower incidence of PTLD in KTx (0% in KTx, 3% in Liver Tx, 11% in Intestinal Tx and 45% in heart Tx, $p<0.0001$), although study years were different (Table 3) (3–5). Notably, two studies have reported a decreased overall incidence of PTLD in EBV viral load monitoring era in pediatric heart and liver Tx. This change is likely owing to better recognition of the presence of elevated EBV viral load and its risk of PTLD (20, 21). Even compared with recent data which showed lowering incidence of PTLD in heart and liver Tx, the overall incidence of PTLD in KTx in the current cohort was the lowest, indicating that organ specific factors could explain the

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better outcome of CHL carrier state in these KTx recipients (22). Potential explanations include: 1. The older age at the time of Tx; 2. Different induction regimens: alemtuzumab is used in KTx while thymoglobulin is most commonly used in heart Tx; 3. Frequent viral load monitoring protocol in KTx program with aggressive titration of immunosuppression; 4. prolonged use of antiviral therapy.

There was no clear benefit on EBV prevention or EBV viral load reduction by antiviral therapy

The fourth potential explanation for better outcomes observed in CHL carriers in this study was the prolonged use of antiviral therapy. Therefore, the impact of antiviral therapy on EBV infection was analyzed in two different ways. To evaluate the potential preventive effect of antiviral therapy, the incidence of primary EBV infection was compared when antivirals were ON during the first 12 months after KTx as per protocol and while OFF therapy during months 12-24 after KTx. A significantly higher incidence of EBV infection was noted while ON antiviral therapy, suggesting a lack of antiviral effect for EBV prevention. Several possible confounding factors regarding a potentially missed antiviral effect should be addressed: 1. There was no “control” incidence of EBV primary infection after KTx in the absence of prophylaxis; 2. Despite a high EBV mismatch (D+/R−) rate (87%, 93/107), not everyone developed EBV infection during the study period. Having said, this experience provides relevant data for potential future randomized controlled studies evaluating the role of antiviral therapy on the incidence of primary EBV infection in the first year after KTx. This study also evaluated the impact of antiviral therapy on the CHL carrier state. Although the dynamics of viral loads can be affected by various clinical reasons, with antiviral therapy being only one of these, the linear model did not identify differences in the overall EBV viral loads in each patient regardless of antiviral therapy. This may be consistent with the fact that antiviral therapy with thymidine kinase inhibitor is only effective when EBV is in lytic phase with replication, but not in different phase such as latency. Similarly, the recently published meta-analysis data showed non-significant preventive effect of antiviral therapy against development of PTLD (8).

Several key strengths of this study warrant mentioning. The transplant database served as a comprehensive source for the 15-year retrospective review including access to all EBV loads obtained under established EBV monitoring protocols allowing assessment of longitudinal dynamics of EBV viral load changes. Additionally, the availability of these resources provided detailed clinical information, including the use of antiviral therapy, allowing assessment of the impact of these agents and other potential exposures (e.g. induction immunosuppression). Finally, the fact that this study was carried out at the same center using the same EBV load assays and EBV load carrier state definitions as previous studies of the CHL carrier state across other pediatric SOT; heart, liver and intestinal transplant recipients, is also a major strength increasing the validity of comparisons between these different pediatric organ recipients.

The limitations of this study should also be addressed including sample size and study design. Although CHP has a moderately large kidney transplant cohort, PTLD in this population is rare accordingly. Therefore, the sample size in this current study at a single

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center, even with 188 KTx recipients, was too small to calculate independent risk factors for PTLD. The second issue is the potential to miss clinically subtle PTLD. While aggressive reduction of immunosuppression has been applied to CHL carriers preemptively, there is no established protocol, such as surveillance imaging and/or biopsies to look for PTLD. Accordingly, it is plausible that clinically occult PTLD was missed. The third limitation is lack of information regarding the correlation of EBV viral loads with intensity of immunosuppression and clinical event such as rejection.

Finally, the impact of antiviral therapy on EBV viral loads from selected 16 CHL carriers was analyzed in univariate manner, while the dynamics of viral load change is multifactorial (e.g. titration of immunosuppression was not considered).

In conclusion, the overall incidence of CHL carrier state and PTLD in KTx is low with serially monitored EBV loads. To understand the risk and different kinetics of EBV viral loads between different organ transplantation will help clinical decision making when KTx recipients require fine tuning of immunosuppression between risks for infection and rejection.

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Table 1

Characteristics of EBV Viral Load Groups in Pediatric Kidney Transplant Recipients.

Number (%) Unless Specified	**Total N=188**	**UVL N=113**	**LVL N=59**	**CHL N=16**	**p value**
Age at Tx (years: Median [IQR])	**12.1**	**12.6***	**13.8^{+}**	**3.9*$^{+}$**	**0.0004**
Post-transplant follow up (years: Median [IQR])	6.9 [4.1–10.5]	6.4 [3.1–9.7]	7.5 [5.9–11.6]	7.3 [4.5–10.9]	0.06
Gender:					
Male	113 (60)	66 (58)	34 (58)	13 (81)	0.20
Female	75 (40)	47 (42)	25 (42)	3 (19)	
Induction Therapy:					0.39
Alemtuzumab	144 (76)	91 (80)	41 (69)	11 (69)	
Anti-thymocyte globulin	11 (6)	5 (4)	5 (8)	1 (6)	
Other or Steroid	34 (18)	17 (15)	13 (22)	4 (25)	
Pre-Tx EBV serology N (%)					
Pre-Tx EBV negative	**107 (57)**	**62 (55)$^{+}$**	**30 (51)***	**15 (94)*$^{+}$**	**0.002**
D−/R−	12 (6)	9 (8)	0 (0)	3 (19)	
D+/R−	93 (49)	52 (46)	29 (48)	12 (75)	
D−/R+	5 (3)	4 (4)	1 (2)	0 (0)	
D+/R+	72 (38)	43 (38)	27 (46)	1 (6)	
Incomplete data	7 (4)	5 (4)	2 (3)	0 (0)	
Incidence of late onset PTLD	1 (0.5)	0 (0)	1 (1.6)	0 (0)	0.34
Incidence of BKV viremia	26 (14)	16 (14)	6 (10)	3 (19)	0.63
Incidence of BK nephropathy	3 (2)	2 (2)	1 (2)	0 (0)	0.81
Incidence of CMV antigenemia/viremia	36 (19)	26 (23)	17 (29)	0 (0)	**0.052**
Pre-Tx CMV negative †	129 (73)	81 (74)*	32 (63)$^{+}$	16 (100)*$^{+}$	**0.012**

Tx: Transplant, IQR: interquartile range, UVL: undetectable viral load carrier, LVL: low detectable viral load carrier, CHL: Chronic EBV high load carrier, D: donor, R: recipient, PTLD: post-transplant lymphoproliferative disorder, BKV: BK virus, CMV: cytomegalovirus. Marks (*$^{+}$) were used when post hoc analysis showed statistical differences.

†Total N=176 due to missing data.

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Table 2

Characteristics of Primary EBV Infection in Pre-Transplant EBV naïve children.

Number (%) Unless Specified	**Total N=56**	**UVL N=11**	**LVL N=30**	**CHL N=15**	**p value**
Age at Tx (years: median [IQR])	5.7 [3.0–13.9]	5.5 [4.6–12.9]	9.1 [2.6–14.5]	3.8 [2.9–7.0]	0.12
Age of Primary EBV infection (years: median [IQR])	7.2 [3.7–16.1]	6.9 [4.4–16.2]	12.0* [5.7–17.0]	4.9* [3.0–7.5]	**0.023**
Timing of primary EBV infection after Tx (months: median [IQR])	7.4 [2.4–26.1]	24.4 [1.4–26.1]	7.1 [2.4–58.9]	7.3 [2.4–16.3]	0.81
EBV serology N (%)					
D+/R−	50 (89)	9 (82)	29 (97)	12 (80)	
D−/R−	5 (9)	2 (18)	0 (0)	3 (20)	
Incomplete data	1 (2)	0 (0)	1 (3)	0 (0)	

Tx: Transplant, IQR: interquartile range, UVL: undetectable viral load carrier, LVL: low detectable viral load carrier, CHL: Chronic EBV high load carrier, D: donor, R: recipient, Mark (*) was used when post hoc analysis showed statistical difference

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Table 3

Characteristics of PTLD and Chronic High Viral Load Carrier across Different Pediatric Solid Organ Transplantation.

Type of Transplant **Number of Children in Each Tx in Reports**	**Kidney** **N=189**	**Liver** **N=196**	**Intestine** **N=166**	**Heart** **N=71**	**p value**
Number of CHL N (%)	16 (8)*+	36 (18)	35 (21)*	20 (28)+	0.0004
Negative Pre-Tx EBV Serology N (%)	15 (94)*	27 (75)	17 (49)*+	20 (100)+	<0.0001
Age at Tx (median)	3.9	ND	ND	1.0	NA
Duration of CHL (Month: Median)	20	12	ND	ND	NA
Onset of CHL after Tx (Month: Median)	7.5	4	6.25	ND	NA
Incidence of PTLD N (%) in CHL	**0 (0)***	**1 (3)+**	**4 (11)#**	**9 (45)*+#**	**<0.0001**
Study year	2000–2014	1997–2007	1994–2007	1989–2004	NA

Tx: Transplant, CHL: Chronic EBV high load carrier, PTLD: post-transplant lymphoproliferative disorder, ND: No data, NA: not applicable or not calculated. Marks (*+, *+#) are used when post hoc analysis showed statistical differences.