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Epidemiology of Epstein-Barr Virus Chronic High Viral Load in Kidney Transplant Recipients

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Background. Epstein-Barr virus (EBV) chronic high viral load (CHVL) may be defined by >16 000 copies/mL whole blood or >200 copies/10^5 peripheral blood mononuclear cells in >50% samples exceeding 6 mo. EBV CHVL has only been characterized in a few small pediatric studies, with heterogeneous results and unclear clinical significance. **Methods.** This single-center observational study evaluated adult and pediatric kidney transplant recipients transplanted between 2010 and 2021 on tacrolimus/mycophenolate-based/prednisone immunosuppression. The primary outcome was EBV CHVL prevalence. Secondary outcomes included recipient characteristics, DNAemia kinetics, and posttransplant lymphoproliferative disorder (PTLD) in recipients with EBV CHVL versus low-grade DNAemia or no DNAemia. **Results.** Five hundred forty-one recipients had a mean follow-up of 4.6 y. Fourteen recipients (2.6%) developed EBV CHVL, 70 (12.9%) had low-grade EBV DNAemia, and 457 (84.5%) had no EBV DNAemia. EBV CHVL was more common in recipients who were Caucasian ($P = 0.04$), younger ($P = 0.04$), received induction immunosuppression ($P = 0.02$), and had high-risk donor–recipient EBV serologic mismatch ($P < 0.0001$). CHVL patients had a higher first viral load ($P = 0.03$), longer time to maximum viral load ($P = 0.02$), and did not achieve sustained DNAemia clearance versus low-grade DNAemia. Three EBV-positive PTLD cases occurred in recipients with a history of EBV DNAemia. PTLD was present in 7.1% (1/14) CHVL versus 2.9% (2/70) low-grade DNAemia patients ($P = 0.002$). EBV DNAemia developed in 32 EBV seronegative recipients (32/59; 54%); clearance was achieved in 70% (14/20) with low-grade DNAemia but no CHVL (0/12; $P = 0.0001$). **Conclusions.** CHVL was uncommon and appeared to occur after primary EBV infection. Future studies should explore other potentially modifiable risk factors for PTLD, including optimal management of EBV DNAemia.

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Posttransplant lymphoproliferative disorder (PTLD) is a potentially devastating complication that affects 1% to 2% of kidney transplant recipients.[1] It occurs in a bimodal distribution with the highest risk in the first year posttransplant, where >90% of cases are associated with Epstein-Barr virus (EBV) DNAemia.[2,3] Although recent data suggest improved overall mortality after PTLD, treatment typically involves stepwise immunosuppression reduction followed by rituximab and chemotherapy

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and may be associated with an increased risk of allograft rejection and treatment-related mortality.[4] Identifying those at high risk of PTLD may facilitate the study of preventative strategies.

Pretransplant EBV seronegative status and posttransplant primary EBV infection have been demonstrated as risk factors for PTLD. As a result, the American Society of Transplantation Infectious Disease Community of Practice (AST-IDCOP) 2019 guidelines recommend posttransplant testing in seronegative recipients to detect primary EBV infection. After initial DNAemia detection, which is recommended until an unspecified "set point" is achieved.[4] However, current strategies of immunosuppression reduction do not appear to impact the viral load set point in the short term and may be followed by allograft rejection.[5,6] Furthermore, there is conflicting evidence on the association between high titer or long-lasting EBV DNAemia and PTLD.[7,8] An EBV chronic high viral load (CHVL) phenotype was described in the context of pediatric heart transplant recipients, defined by the presence of a high viral load exceeding >16 000 copies/mL in whole blood samples or >200 copies/10^5 peripheral blood mononuclear cells in >50% of samples for at least 6 mo, due to concern for a signal of increased risk for PTLD.[9]

To date, the EBV CHVL phenotype has only been characterized in a few small pediatric studies with heterogeneous results. Among kidney transplant recipients, reported prevalence rates vary from 8% to 47% and is more prevalent in younger and EBV seronegative recipients.[10-14] Characterization of the natural history of EBV CHVL has been similarly mixed, with Ladfors et al[10] describing persistent DNAemia despite immunosuppression reduction, whereas Yamada et al[12] described resolution in almost all patients. Whereas several studies have reported low to negligible risk of PTLD among pediatric kidney transplant recipients, high EBV viral load exceeding about 60 000 copies/mL correlated with increased probability of developing PTLD in an Italian cohort.[11-15] It is not known whether results may be extrapolated to adult kidney transplant recipients who often receive different immunosuppression protocols.

To address these questions and research priorities highlighted by recent AST guidelines, we studied a consecutive cohort of adult kidney transplant recipients maintained on tacrolimus (Tac), mycophenolic acid (MPA), and prednisone, and with long-term follow-up, to describe the epidemiology and natural history of EBV CHVL.

MATERIALS AND METHODS

Study Design and Population

The study received ethics approval from the University of Manitoba Health Research Ethics Board (HS24719, H2021:095). This single-center cohort study consisted of 560 consecutive adult and pediatric kidney transplant recipients from January 2010 to May 2021 from Transplant Manitoba on triple maintenance immunosuppression with Tac, MPA, and prednisone. Recipients were excluded if there was missing pretransplant EBV serology (n = 2), there was pretransplant EBV DNAemia (n = 3), or primary nonfunction (n = 16). Induction therapy was used in 65% of recipients, including thymoglobulin (28%) or basiliximab (37%).

EBV Screening, Treatment, and Monitoring

Quantitative EBV polymerase chain reaction (PCR) is performed in whole blood samples and processed by a single laboratory, Cadham Provincial Laboratory. Routine EBV screening is only performed in recipients with high-risk donor–recipient EBV serologic mismatch (donor seropositive and recipient seronegative). The Transplant Manitoba EBV screening protocol was modified in March 2020 to align with AST-IDCOP 2019 guidelines with less intensive monitoring than previously done.[4] The standard protocol included EBV viral PCR testing at 1 wk posttransplant and then monthly for 1 y. After treatment of rejection, EBV PCR was checked once. In the revised protocol, testing is done monthly till 6 mo posttransplant, every 3 mo till 1 y posttransplant, and then every 6 mo till 2 to 3 y posttransplant. After treatment of rejection, EBV PCR is checked once 3 mo after treatment. In both protocols, EBV PCR is checked if clinically indicated.

Following EBV DNAemia detection, maintenance immunosuppression is reduced in a standardized stepwise manner, starting with the antimetabolite, which is dose-reduced until discontinued, followed by the calcineurin inhibitor. The degree and extent of immunosuppression reduction are considered on a case-by-case basis and approaches used in our center detailed in the Supplemental Methods (**SDC,** http://links.lww.com/TXD/A620).

HLA Typing and Eplet Molecular Mismatch Identification

Class II HLA typing (HLA-DRβ1/3/4/5 and HLA-DQα1/β1) was done using sequence-specific oligonucleotide probes or sequence-specific primer technology (LABType HD SSO, Micro SSP, One Lambda). HLAMatchmaker software (HLA DRDQDP Matching version 2.2) was used to determine the eplet mismatch for each HLA-DR or HLA-DQ molecule individually. The single-molecule eplet mismatch was used to categorize individuals into 3 alloimmune risk groups (low, intermediate, or high) using previously described thresholds (Supplemental Methods, **SDC,** http://links.lww.com/TXD/A620).[16-18]

Outcomes

The primary outcome was the prevalence of EBV CHVL defined by the presence of a viral load >16 000 copies/mL whole blood or >200 copies/10^5 peripheral blood mononuclear cells in >50% of samples for at least 6 mo.[9,19] We described baseline demographics, immunosuppression, DNAemia kinetics, outcomes of PTLD, and other viral DNAemias in recipients with CHVL compared with those with low viral load or no DNAemia. EBV DNAemia clearance was defined as 2 negative whole-blood EBV PCR samples at least 1 wk apart. In a secondary analysis, these characteristics and outcomes were reported by EBV donor–recipient serologic matches.

Statistics

Analyses were conducted using JMP Pro (version 15.0). Descriptive statistics were done with categorical variables presented as frequency and percentage and tested using the chi square test or the Fisher exact test. Continuous variables were presented as median and interquartile range and tested using the Wilcoxon rank-sum test for nonparametric data. We also a conducted sensitivity analysis restricted to EBV seronegative recipients at the time of transplant who developed primary EBV infection posttransplant, to compare the frequency of viremia clearance in those with CHVL versus low viral load. *P* values of <0.05 were considered significant.

Guidelines

The study complies with the Strengthening the Reporting of Observational Studies in Epidemiology checklist.

RESULTS

Study Population

The final study population consisted of 541 kidney transplant patients, including 511 adult patients, with a median age 52 y (interquartile range [IQR], 38–62) and a mean follow-up of 4.6 y. The distribution of the alloimmune risk score based on the HLA eplet mismatch was 109 (20%) low, 202 (37%) intermediate, and 230 (43%) high risk. The baseline demographics of the final study population (n = 541) were representative of the overall consecutive cohort (n = 560). There were 14 patients (2.6%) who developed EBV DNAemia with CHVL, 70 (12.9%) who had low-grade EBV DNAemia, and 457 patients (84.5%) who never experienced detectable EBV DNAemia (Figure 1).

Baseline Demographics by EBV Viral Load Phenotype

Recipients with EBV CHVL were more likely to be younger at the time of transplant (32.3 y; IQR, 14.3–57.0), with 6 (43%) pediatric recipients at the time of transplant. A greater proportion of recipients with EBV CHVL were Caucasian (86%). Among EBV groups, there was no difference in donor type, alloimmune risk category, or cytomegalovirus serological status requiring antiviral prophylaxis as per institutional protocol. High-risk EBV serologic mismatch was more common in those who developed EBV CHVL (85.7%) compared with those with EBV low-grade DNAemia (28.6%) or those without EBV DNAemia (4.8%; $P < 0.0001$). Although induction immunosuppression was more common in those who developed any EBV DNAemia, there was no association between thymoglobulin and development of EBV CHVL (Table 1).

Viral Load Kinetics

There was no difference in time to first viral load for EBV CHVL versus low-grade DNAemia (0.30 [IQR, 0.16–0.52] versus 0.70 [IQR, 0.08–3.24] y; $P = 0.21$). Recipients with EBV CHVL tended to have a greater first viral load titer than those with EBV low-grade DNAemia (1520 [IQR, 288–3675] versus 288 [288–1350] copies/mL; $P = 0.03$). Maximum viral load was greater in the EBV CHVL group ($P < 0.0001$) as per the definition of CHVL, and this maximum viral load was

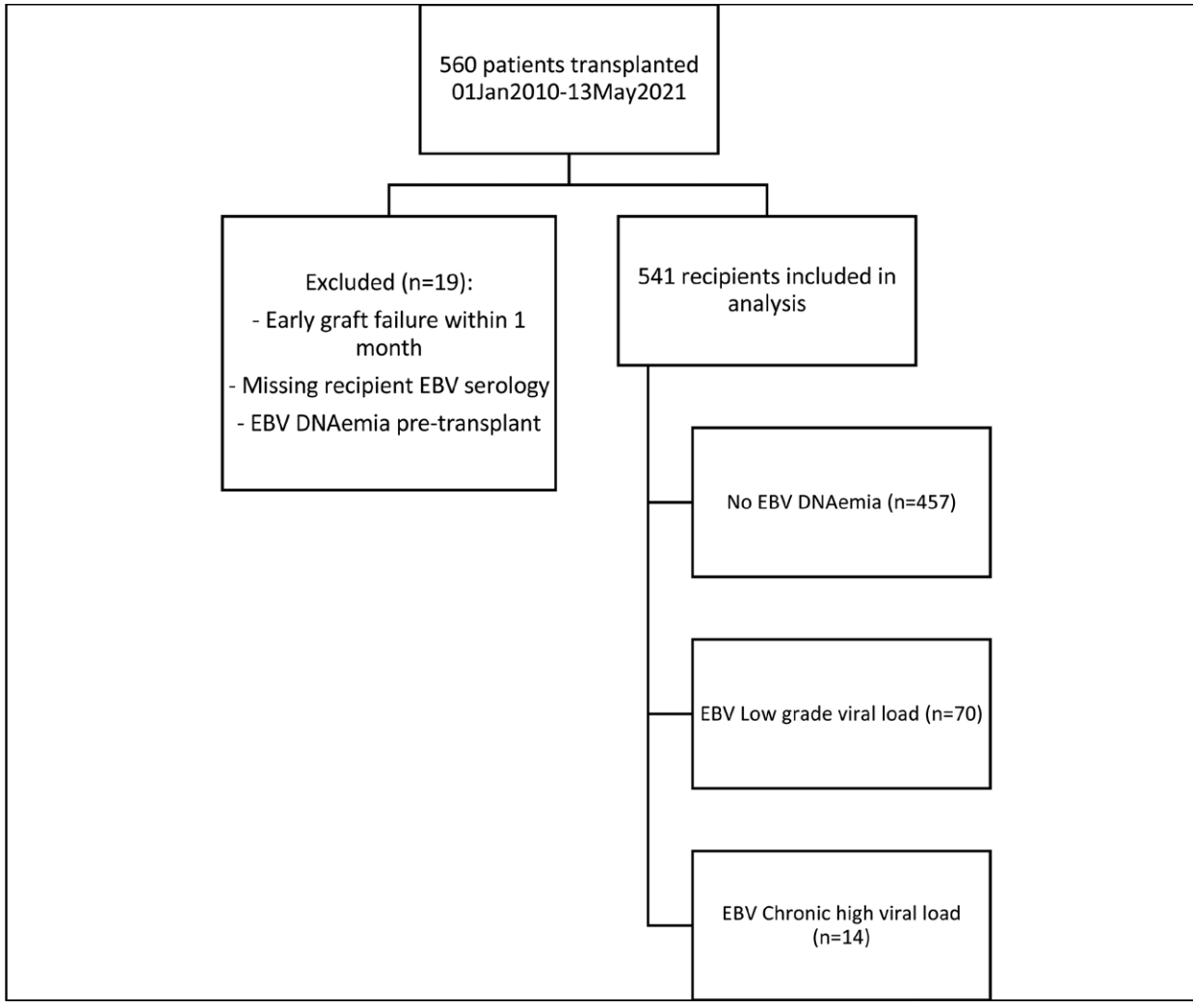

FIGURE 1. CONSORT diagram for EBV cohort. CONSORT, Consolidated Standards of Reporting Trials; EBV, Epstein-Barr virus.

TABLE 1.

Characteristics of adult kidney transplant recipients by EBV DNAemia status (N = 541)

Characteristic	No EBV DNAemia (N = 457)	Low-grade viral load (N = 70)	Chronic high viral load (N = 14)	*P*
Median follow-up time from transplant, y	3.9 (1.8–7.0)	3.9 (2.0–6.1)	4.5 (3.3–7.1)	0.57
Recipient age at transplant, y	**52.2 (40.3–61.7)**	**49.7 (35.7–62.9)**	**32.3 (14.3–57.0)**	**0.04**
Male, n (%)	283 (62.1%)	40 (57.1%)	8 (57.1%)	0.68
Caucasian, n (%)	**268 (58.2%)**	**35 (50.0%)**	**12 (85.7%)**	**0.04**
Living donor, n (%)	209 (45.7%)	33 (47.1%)	8 (57.1%)	0.71
Alloimmune risk category, n (%)				0.62
Low	94 (20.6%)	12 (17.1%)	3 (21.4%)	
Intermediate	169 (37.0%)	30 (42.9%)	3 (21.4%)	
High	194 (42.5%)	28 (40.0%)	8 (57.1%)	
High CMV risk ($D^+ R^-$) or R^+ and thymoglobulin (N = 499), n (%)	228 (54.7%)	40 (61.5%)	8 (57.1%)	0.59
High EBV risk $D^+ R^-$, n (%)	**22 (4.8%)**	**20 (28.6%)**	**12 (85.7%)**	**<0.0001**
EBV D/R match, n (%)				**<0.0001**
$D^+ R^-$	**22 (4.8%)**	**20 (28.6%)**	**12 (85.7%)**	
$D^+ R^+$	**381 (83.4%)**	**42 (60.0%)**	**2 (14.3%)**	
$D^- R^+$	**42 (9.2%)**	**6 (8.6%)**	**0**	
$D^- R^-$	**12 (2.6%)**	**2 (2.9%)**	**0**	
Induction, n (%)	**287 (62.8%)**	**56 (80.0%)**	**10 (71.4%)**	**0.02**
Thymoglobulin, n (%)	**127 (27.8%)**	**25 (35.7%)**	**2 (14.3%)**	**0.24**
Thymoglobulin vs basiliximab (N = 353) , n (%)	127 (44.3%)	25 (44.6%)	2 (20.0%)	0.35
Thymoglobulin vs no induction (N = 342) , n (%)	**127 (42.8%)**	**25 (64.1%)**	**2 (33.3%)**	**0.035**

Chronic high viral load: >50% of DNAemia samples >16 000 copies/mL >E 6 mo.
Data presented as median (IQR) or n (%) within the EBV group unless otherwise stated.
The Kruskal-Wallis test or the chi square test for *P* values.
High CMV risk if D^+/R^- or R^+ with thymoglobulin (ATG) induction.
Bold values indicate statistical significance.
CMV, cytomegalovirus; D^+, donor seropositive; D^-, donor seronegative; IQR, interquartile range; R^+, recipient seropositive; R^-, recipient seronegative.

TABLE 2.

Characteristics and outcomes of recipients with EBV DNAemia (N = 84)

Characteristic	Low-grade viral load (N = 70)	Chronic high viral load (N = 14)	*P*
Time to first viral load, y	0.70 (0.08–3.24)	0.30 (0.16–0.52)	0.21
First viral load magnitude, copies/mL	**288 (288–1350)**	**1520 (288–3675)**	**0.03**
Median follow-up time from first viral load, y	**2.2 (1.1–3.5)**	**4.1 (3.0–5.5)**	**0.0005**
Time to maximum viral load, y	**1.3 (0.18–3.8)**	**3.3 (2.2–4.8)**	**0.02**
Maximum viral load magnitude, copies/mL	**811 (288–3685)**	**96 550 (47 750–267 500)**	**<0.0001**
Cleared DNAemia, n (%)	**30 (42.9%)**	**0**	**0.002**
Time to DNAemia clearance, y (N = 30)	**0.24 (0.11–0.57)**		
PTLD, n (%)	**2 (2.9%)**	**1 (7.1%)**	**0.002**
Time to PTLD from transplant, y (N = 3)	2.3 (0.60–4.0)	0.70 (0.70–0.70)	1.0
Time to PTLD from first viral load, y (N = 3)	0.003 (–0.04 to 0.04)	0.35 (0.35–0.35)	0.67

Chronic high viral load: >50% of DNAemia samples >16 000 copies/mL >E 6 mo.
Data presented as median (IQR), or n (%) within the EBV group unless otherwise stated.
The Kruskal-Wallis test or the chi square for *P* values.
Bold values indicate statistical significance.
EBV, Epstein-Barr virus; IQR, interquartile range; PTLD, posttransplant lymphoproliferative disorder.

reached later in the EBV CHVL group (3.3 [IQR, 2.2–4.8] versus 1.3 [IQR, 0.18–3.8] y, $P = 0.02$). EBV DNAemia clearance was achieved in 43% of those with EBV low-grade DNAemia, but none of the EBV CHVL group ($P = 0.002$) despite longer median follow-up ($P = 0.0005$; Table 2). There was no association between EBV CHVL and the presence of either cytomegalovirus or BK virus DNAemia (data not shown).

Posttransplant Lymphoproliferative Disorder

Three cases of biopsy-proven EBV-positive PTLD were identified during the study period, with 2 patients having pretransplant EBV mismatch (D^+/R^-) and 1 patient who was not mismatched (D^+/R^+). There was 1 pediatric patient at the time of transplant with a PTLD diagnosis. EBV-positive diffuse large B-cell lymphoma was present in 7.1% (1/14) of CHVL patients versus 2.9% (2/70) of low-grade DNAemia patients ($P = 0.002$). There was no difference in time to PTLD diagnosis from transplant or first detected DNAemia (Table 2).

EBV Donor–Recipient Serologic Match

There were 532 donor–recipient pairs with complete serology available. Recipients with high-risk donor–recipient EBV serologic mismatch (donor seropositive, recipient seronegative) had higher maximum viral loads ($P = 0.01$) and a higher rate of PTLD ($P = 0.01$) compared with other donor–recipient EBV serologic combinations (**Table S1, SDC,** http://links.lww.com/TXD/A620).

Sensitivity Analysis

Among 59 EBV-seronegative recipients at the time of transplant, EBV DNAemia was detected in 32 recipients (32/59; 54%). EBV DNAemia clearance was achieved in 14 of 20 (70%) recipients with low-grade EBV DNAemia, but none of those of the EBV CHVL group (0/12; 0%; $P = 0.0001$).

DISCUSSION

This is the first study to describe the EBV CHVL phenotype in a cohort that includes adult kidney transplant recipients receiving modern Tac/MPA/prednisone immunosuppression. EBV CHVL was observed in 2.6% of recipients and occurred more commonly in those who were younger, Caucasian, received induction immunosuppression, and with pretransplant high-risk donor–recipient EBV serologic mismatch. Among recipients with detectable EBV DNAemia, those who developed CHVL tended to have a higher first viral load followed by a longer time to reach maximum viral load. Moreover, EBV DNAemia clearance did not occur in any recipients with CHVL. All EBV-positive PTLD cases occurred in those with EBV DNAemia, with a trend toward an increased rate of PTLD in the CHVL group, albeit with a very small number of cases.

In this novel description of EBV CHVL in adult kidney transplant recipients, we observed a lower prevalence of CHVL than previously described.[10-14] This was anticipated as younger age and pretransplant EBV seronegative status are well-described risk factors for CHVL in the pediatric literature. Although we similarly identified these risk factors, they were less common than in pediatric populations where up to half of recipients may be EBV seronegative before transplant, in contrast to only 12.6% of our cohort. Although seropositive individuals are protected against primary EBV infection posttransplant, this assumption cannot be universally applied as adults in the developed world achieve EBV seroprevalence later in life, with 90% seroprevalence at 40 versus 5 y old in the developing world.[20] It is possible that the induction immunosuppression protocol of our center based on alloimmune risk score may have contributed to lower CHVL prevalence because the use of any induction immunosuppression correlated with increased CHVL. Indeed, 35% of recipients in our cohort did not receive any induction immunosuppression because of the low alloimmune risk score/low HLA eplet mismatch at the time of transplant, an approach not widely used among transplant centers.[18] Taken together, these risk factors suggest that the establishment of CHVL is more likely following primary EBV infection posttransplant and supports current guideline recommendations for posttransplant EBV viral load surveillance in recipients at increased risk of primary infection.

After CHVL is established, ongoing viral surveillance with preemptive interventions is not currently recommended because of uncertainty in best immunosuppression management practices.[4] Variable DNAemia clearance rates are described in pediatric cohorts, with considerable study heterogeneity, including types of solid organ transplant and approaches to EBV monitoring and immunosuppression management. No cases of CHVL in our cohort achieved DNAemia clearance despite protocolized immunosuppression

reduction over 4.6 y of follow-up. Ladfors et al[10] similarly described persistent EBV DNAemia in pediatric kidney transplant recipients, with a median DNAemia duration of 2 y despite immunosuppression reduction. Notably, a case series of pediatric liver transplant recipients reported persistent DNAemia even after withdrawal of all immunosuppression, an approach that would not be feasible in kidney transplant recipients.[5] Although our center uses a standardized approach to managing EBV DNAemia, the effects of real-world immunosuppression modification on subsequent CHVL development or clearance were not captured and should be assessed in future time-dependent analyses. Recipients with CHVL may be exposed to greater immunosuppression reduction in response to higher viral loads or prolonged periods of DNAemia. Future studies should also explore any modifying effect of immunosuppression reduction in the context of potential adverse effects (eg, rejection). None of our patients received rituximab, facilitating this natural history description of EBV DNAemia managed per current conventions.

There is a need to identify predictors of EBV DNAemia clearance. It is possible that EBV seroconversion after primary infection may be a predictor of DNAemia clearance, but this was not tested in our cohort. Moran et al[14] also described an association of HLA-A*02 with CHVL development, whereas HLA-B*08 was associated with DNAemia clearance. Further studies are also needed to explore the role that viral factors play in PTLD pathogenesis. During the primary EBV infection, circulating B cells are infected, leading to persistent infection in a nonreplicative latent form. This latent form is sustained by several EBV-latent genes, including LMP1, LMP2, EBNA1, EBNA2, EBNA-LP, and EBNA3A/3B/3C, all of which are expressed in EBV-related PTLD.[21] EBV is categorized into 2 strains, A and B, which are further subdivided on the basis of minor genetic differences, and some studies have suggested that regions of increased variation exist in EBV genomes isolated from transplant recipients with PTLD.[22] Understanding the presence of strains with varying oncogenic potential is critical for risk assessment, devising effective treatment strategies, and predicting disease progression.

There was a trend suggesting CHVL may be associated with a higher prevalence of EBV-positive PTLD than low-grade DNAemia, albeit in a very limited number of cases. Given the low number of PTLD cases, it is possible our study was underpowered to characterize differences in PTLD rates between the CHVL and low-grade viral load groups. However, the overall prevalence of PTLD observed was consistent with expected rates among kidney transplant recipients receiving modern immunosuppression. An Italian cohort of 304 pediatric kidney transplant recipients reported increased PTLD risk in recipients with a viral load exceeding 60 000 copies/mL but without criteria for the duration of DNAemia.[15] As there is presently no consensus definition for EBV CHVL, our study cohort was characterized using one of the more commonly used definitions.[9,19] It is possible that a revised definition of CHVL may better correlate with PTLD risk.

Strengths of our study include a large cohort of recipients on modern Tac/MPA/prednisone maintenance immunosuppression with complete viral load data, a uniform approach to immunosuppression reduction, and a long duration of follow-up. This is the first study to describe CHVL in adult kidney transplant recipients, and it is the largest study conducted on kidney transplant recipients describing the natural history of EBV CHVL. The mean follow-up time of 4.6 y was sufficiently long to observe EBV DNAemia and EBV-associated PTLD, where the majority of cases occur early posttransplant. All EBV viral load testing was performed in a single provincial laboratory, minimizing interlaboratory variability and allowing for the assessment of dynamic viral load trends over time.[23,24]

Due to the relatively small sample size and associated risk of type II error, risk quantification should be interpreted with caution and validated in independent cohorts. A small sample size also precluded multivariable analyses, and future studies should explore specific risks of PTLD specific to EBV CHVL. This was a retrospective study and residual unmeasured confounding effects are possible. MPA dose and TAC trough means and coefficient of variation were not available to ascertain the effect of baseline immunosuppression on the development of EBV DNAemia. Although treatment records were not individually reviewed to directly ascertain immunosuppression reduction, the Transplant Manitoba program follows a uniform management protocol in a shared clinical practice. Transplant Manitoba adopted a reduced frequency EBV screening protocol in March 2020, but this is not anticipated to impact the results given ample opportunities for EBV DNAemia testing. Future studies are needed to ascertain the association between EBV CHVL phenotype and long-term graft outcomes.

In conclusion, EBV CHVL was not common among kidney transplant recipients and appeared to occur in the context of primary EBV infection posttransplant. All cases of PTLD occurred in recipients with EBV DNAemia, with a similar prevalence noted in those with low-grade viral load and CHVL. Future studies should explore other potentially modifiable risk factors for PTLD development, including optimal management of EBV DNAemia.

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