American Journal of Transplantation 2014; 14: 1619–1629
Wiley Periodicals Inc.

doi: 10.1111/ajt.12703

Epstein–Barr Virus Infection in Adult Renal Transplant Recipients

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Epstein–Barr virus (EBV) DNAemia in the first year posttransplantation has been studied extensively. There is a paucity of information on prevalence and sequelae of EBV infection in adult renal transplantation beyond the first year. This single-center study examines the relationship between EBV DNAemia and demographic, immunosuppressive, hematologic and infection-related parameters in 499 renal transplant recipients between 1 month and 33 years posttransplant. Participants were tested repeatedly for EBV DNAemia detection over 12 months and clinical progress followed for 3 years. Prevalence of DNAemia at recruitment increased significantly with time from transplant. In multivariate adjusted analyses, variables associated with DNAemia included EBV seronegative status at transplant ($p = 0.045$), non-White ethnicity ($p = 0.014$) and previous posttransplant lymphoproliferative disease (PTLD) diagnosis ($p = 0.006$), while low DNAemia rates were associated with mycophenolate mofetil use ($p < 0.0001$) and EBV viral capsid antigen positive Epstein–Barr nuclear antigen-1 positive serostatus at transplant ($p = 0.044$). Patient and graft survival, rate of kidney function decline and patient reported symptoms were not significantly different between EBV DNAemia positive and negative groups. EBV DNAemia is common posttransplant and increases with time from transplantation, but EBV DNAemia detection in low-risk (seropositive) patients has poor specificity as a biomarker for future PTLD risk.

Keywords: Epstein–Barr virus, immunosuppression, PTLD, renal transplantation

Abbreviations: ATG, anti-thymocyte globulin; CI, confidence interval; CMV, cytomegalovirus; EBNA, Epstein–Barr nuclear antigen-1; EBV, Epstein–Barr virus; ECOG, Eastern Cooperative Oncology Group; eGFR, estimated GFR; HR, hazard ratio; HVL, high viral load carriage; IQR, interquartile range; MMF, mycophenolate mofetil; NDNA, no detectable DNA; OR, odds ratio; PCR, polymerase chain reaction; PDNA, persistent DNAemia; PTLD, posttransplant lymphoproliferative disease; TDNA, transient detection; VCA, viral capsid antigen

Received 04 June 2013, revised 15 January 2014 and accepted for publication 05 February 2014

Introduction

Current guidelines recommend screening for Epstein–Barr virus (EBV) DNA in blood in high-risk recipients for 1 year after transplantation (1–4). In these patients, preemptive reduction of immunosuppression and treatments such as rituximab may reduce the incidence of early EBV-related disease and posttransplant lymphoproliferative disease (PTLD) (5–7). In adult kidney transplant recipients, PTLD is a late event, with median time to presentation in our population of 74 months (8,9). The prevalence of EBV DNAemia in the late posttransplant period is unclear, and the logistics of screening and monitoring challenging. In those with late detectable EBV DNA and no current evidence of clinical disease or PTLD, the implications of DNAemia are uncertain. Therefore, we performed a prospective observational study in 499 adult kidney transplant recipients, clinically stable at recruitment, of which 92% were beyond the first posttransplant year. We conducted a cross-sectional analysis of the prevalence of EBV DNAemia at recruitment, and subsequent patterns of DNAemia over a 1-year period. Factors associated with EBV DNAemia, patient and graft survival, and incidence of PTLD were analyzed after 3 years of follow-up.

Methods

Patient group

Inclusion criteria were age >18 years and presence of a functioning kidney graft. All eligible patients at Manchester Royal Infirmary were invited by letter to join the study. Those who responded positively were recruited between February 22, 2010 and September 13, 2010. Participants were between 1 month and 33 years posttransplantation. Since 2004 our unit immunosuppression protocol included basiliximab induction therapy,

tacrolimus maintenance, with addition of mycophenolate mofetil (MMF) and prednisolone depending on clinical circumstance. Valganciclovir prophylaxis was given for 100 days to cytomegalovirus (CMV) donor positive–recipient negative cases, or those who received lymphocyte depleting agents. BK screening was not routinely performed, but prompted by clinical indication.

Sampling and polymerase chain reaction

Whole blood EDTA was analyzed for the presence of EBV DNA at recruitment and at 3-month intervals for 1 year. DNA extracts were batched and stored at −80°C, and a quantitative real-time polymerase chain reaction (PCR) assay with the target gene BNRF-p143 (10) was used with the Applied Biosystems 7500 Fast Real-Time PCR (Thermo Fisher Scientific Inc., Paisley, UK). The threshold of sensitivity for detection of EBV DNA was 1000 copies/mL blood. Samples with no detectable EBV DNA, or concentrations measured at <1000 copies/mL, were classified as undetectable. Following the study we obtained the International Standard for EBV DNA from the UK National Institutes for Biological Standards and Control (NIBSC code: 09/260; version 3.0; 11/04/2013; Potters Bar, UK). The International Standard and our plasmid standard (Eurofins MWG Operon, Ebersberg, Germany) diluted in parallel curves (Ct value vs. dilution), and triplicate titrations were averaged to derive the conversion factor of 1 International Standard unit = 0.5 copies of our EBV PCR plasmid standard.

Blood samples were taken at recruitment for analysis of EBV serostatus (presence of IgG antibodies to EBV viral capsid antigen [VCA] and Epstein–Barr nuclear antigen-1 [EBNA] antigens [U/mL]) using the DiaSorin S.P.A LIAISON (Saluggia, Italy) analyzer. Pretransplant EBV serostatus was obtained from medical records or from analysis of frozen serum samples from the Regional Tissue Typing laboratory. Demographic, transplant and immunosuppressive details were obtained from medical records at the time of recruitment.

Symptoms assessment

All recruited individuals were invited to complete a survey of clinical symptoms associated with EBV and PTLD including fever, night sweats, weight loss, loss of appetite, sinus congestion, anorexia, swollen glands, sore throat, loss of appetite and abdominal pain (11). Physician assessed Karnofsky and Eastern Cooperative Oncology Group (ECOG) scores were completed at baseline for each individual.

Patterns of EBV DNAemia

Prevalence of EBV DNAemia was calculated for the study population at recruitment (baseline), and then patterns of EBV DNAemia over time were analyzed for recruited individuals with three or more samples, obtained over a period of greater than 6 months (446 individuals). We assigned individuals to classes of no detectable DNA (NDNA), transient detection (TDNA)(<75% samples with detectable DNA), persistent DNAemia (PDNA)(≥75% samples with detectable DNA) and high viral load carriage (HVL) (≥3 samples over >6 months with ≥10 000 copies/mL DNA) (12,13). Individuals with <3 samples (n = 53) were excluded from this analysis.

Analysis of factors associated with EBV infection

Factors predicted to be associated with the presence of EBV DNAemia included age and time from transplant, EBV recipient serostatus at transplantation, immunosuppressive agent exposure, rejection history, biochemical and hematological parameters, including white cell differential and renal graft function, and posttransplant complications including opportunistic infections and nonmelanoma skin cancer. Treated CMV infection included those admitted, or prescribed intravenous ganciclovir, for severe infection. BK viremia included cases detected after testing for clinical indication.

Statistics

Groups were described using the median and interquartile range (IQR). Group comparisons used Fisher's exact tests for categorical variables and Mann–Whitney for ordinal data sets. Statistical analyses were performed using SPSS (IBM SPSS Statistics 20; IBM United Kingdom Limited, Hampshire, UK).

Logistic regression was used to estimate the odds ratios (OR) and 95% confidence interval (CI) for dichotomized factors, both unadjusted and in a multivariable-adjusted model, including time from transplant as a cubic function (the functional form was selected on the basis of the Akaike information criteria in a preliminary analysis), ethnicity, gender, immunosuppressive therapy and previous PTLD diagnosis.

These analyses were conducted in R v2.15 (R Core Team, Vienna, Austria; http://www.R-project.org/).

Participants were followed until February 22, 2013, with subsequent analysis of patient and graft survival, graft function and development of PTLD in relation to EBV DNA detection at recruitment and subsequent EBV DNAemia patterns. Kaplan–Meier survival curves and log-rank tests were used to analyze differences between groups. Individuals with a history of PTLD prior to recruitment were excluded from analyses of PTLD development during the follow-up period (GraphPad Prism version 5.00 for Windows; GraphPad Software, San Diego CA; http://www.graphpad.com).

Ethical approval

Research Ethics Committee review and approval for the study (Greater Manchester NEREC 09/H1013/71) was obtained. Written informed consent was obtained from all subjects participating in the study.

Results

We recruited 499 (56%) individuals from an eligible population of 894 patients. Demographic and transplant details of the recruited population and baseline demographics of nonrecruited patients are shown in Table 1. The median follow-up time of the study population following recruitment was 995 days (IQR 946–1064, range 26–1096 days).

EBV serostatus

At transplantation, 54/451 (12%) recruited individuals with available serology were EBV seronegative (VCA negative EBNA negative) and 397 (88%) seropositive (VCA and/or EBNA positive). At recruitment, 42 of the 54 (78%) seronegative recipients had evidence of seroconversion (VCA or EBNA IgG antibodies), 9 (17%) remained seronegative and 3 (6%) had no samples tested. During the study period (1 year) six of the nine seronegative patients had no detectable DNAemia, including three who remained VCA and EBNA IgG negative, one with no end study serology and two who developed VCA IgG but no anti-EBNA antibodies. A further three of the nine individuals had detectable and PDNA, including two with chronic high viral loads. All three developed VCA antibodies and two-thirds EBNA antibodies. Among 84 individuals with VCA positive EBNA negative serology at transplantation, 50 (60%) remained EBNA negative, 23 (27%) acquired EBNA

Table 1: Demographics of the study population (n = 499) and the nonrecruited population (n = 395)

Characteristics	Patients recruited to the study (n = 499), n %[1]	Nonrecruited patients (n = 395), n %	p
Mean age (diagnosis) (SD) (range)	51.4 (13) (20–81)	47 (14) (18–83)	<0.0001
Median time from transplantation (years) (IQR)	7 (3–12)	6 (2–12)	0.47
Male gender	310 (62)	242 (61)	0.85
Female gender	189 (38)	153 (39)	0.85
Ethnicity			
White	465 (93)	320 (81)	<0.0001
Black	8 (2)	3 (1)	0.36
Asian	26 (5)	72 (18)	<0.0001
Transplant history			
First transplant	404 (81)	–	–
Transplant number 2 and 3	94 (19)	–	–
Living donor	107 (21)	–	–
Deceased donor	392 (79)	–	–
Median creatinine (IQR)	133 (107–173)	–	–
Median eGFR (IQR)	44.3 (33–57)	–	–
Time of transplantation serology			
EBV recipient seronegative	54 (12)	–	–
EBV recipient seropositive	397 (88)	–	–
EBV data missing	48	–	–
CMV recipient seronegative	146 (50)	–	–
CMV recipient seropositive	148 (50)	–	–
CMV data missing	205	–	–
Cause of ESRD			
Diabetes mellitus	58 (12)	–	–
Polycystic	85 (17)	–	–
GN/autoimmune	142 (28)	–	–
Reflux/CPN/Cong.	109 (22)	–	–
Hypertension/renovascular	29 (6)	–	–
Other/unknown	76 (15)	–	–
Basiliximab induction	225 (45)	–	–
Treated rejection	105 (21)	–	–
ATG (rejection)	32 (6.4)	–	–
PTLD diagnosis prior to recruitment	22 (4)	–	–
Treated CMV disease	68 (14)	–	–
Current diabetes mellitus	95 (19)	–	–
Immunosuppression at recruitment			
Tacrolimus	278 (56)	–	–
Ciclosporin	127 (25)	–	–
CNI containing	405 (81)	–	–
Monotherapy	109 (22)	–	–
Dual therapy	276 (56)	–	–
Triple therapy	110 (22)	–	–
Azathioprine	110 (22)	–	–
Mycophenolate mofetil	205 (41)	–	–
Sirolimus	12 (2)	–	–
Prednisolone	265 (53)	–	–

ATG, anti-thymocyte globulin; CMV, cytomegalovirus; CNI, calcineurin inhibitor; Cong., congenital; CPN, chronic pyelonephritis; EBV, Epstein–Barr virus; eGFR, estimated GFR; ESRD, end-stage renal disease; GN, glomerulonephritis; IQR, interquartile range; PTLD, posttransplant lymphoproliferative disease; SD, standard deviation.
[1]Number and percentage are given for each characteristic unless otherwise stated.

antibodies, 8 (10%) lost VCA antibodies and 3 (4%) had no available serology at recruitment.

Symptoms assessment

Results from the survey of clinical symptoms associated with EBV and PTLD, including fever, night sweats, weight loss, loss of appetite, sinus congestion, anorexia, swollen glands, sore throat, loss of appetite and abdominal pain showed no detectable differences between those patients with persistently undetectable, persistently detectable or high EBV viral loads. Overall patient survival was significantly lower in those with symptoms at recruitment including weight loss (86.4% vs. 96.3%, p = 0.002), loss

Table 2: Patterns of EBV DNAemia

Pattern of DNAemia	N (%), n = 446
No detectable DNA (NDNA)	234 (52)
Transient detection (TDNA)	139 (31)
Persistent DNAemia (PDNA)	42 (9)
High viral load carriage (HVL)	31 (7)

Patterns of Epstein–Barr virus (EBV) DNAemia observed in 446 individuals with three or more samples analyzed for EBV DNA over a period greater than 6 months.

of appetite (90.7% vs. 96%, $p = 0.011$), ECOG performance status ≥ 2 (88% vs. 96.1%, $p < 0.0001$) and Karnofsky score <80 (88.4% vs. 96.3%, $p < 0.0001$).

Prevalence and patterns of EBV DNAemia

EBV DNA was detectable (>1000 copies/mL blood) in 153/499 (31%) individuals and undetectable in 346 (69%) at baseline assessment. Viral loads were >1000 copies/mL in 94 (19%), >10 000 in 47 (9%) and ≥100 000 in 12 (2%) individuals. During the 1-year study period, a total of 232/499 (46%) individuals had one or more positive samples. A median number of 4 samples, range 3–10, was obtained from each patient with a median time of 113 days (IQR = 70–138) between sampling points. Patterns of DNAemia are shown in Table 2. In the 53/499 patients with insufficient samples for pattern analysis, reasons for inadequate sampling included death (n = 10), return to base hospital (n = 23), return to dialysis (n = 2) and missed sampling/lost to follow-up (n = 18).

The proportions of patients with detectable EBV DNA at recruitment who also had detectable DNA at the end of the sampling period are shown in Table 3. The higher the initial viral load, the more likely the individual was to have a detectable viral load at the final sampling point (OR = 5.9, 95% CI 2.7–12.8, $p < 0.0001$), for those >10 000 (log 4) copies/mL versus 1000 (log 3) copies/mL.

Influence of time from transplant on EBV DNA detection

The prevalence of EBV DNAemia increased significantly with time from transplant (Figure 1A) from 16% (6/38) in year 1, to 40% (44/111) in the 10th to 14th years, to 66% (19/29) in the 20th to 24th years, with an OR of 2.8 (CI 1.9–4.1) for DNAemia for those more than 10 years posttransplant versus those <10 years ($p < 0.0001$). A fall in DNAemia prevalence was noted in the small number of individuals transplanted for more than 25 years. A similar pattern of increasing prevalence with time was seen in those with transient EBV detection ($p = 0.001$) while proportions of those with persistent detection and chronic HVL increased over the first 20 years, but remained less than 15% of all individuals (Figure 1B). Proportions of those with persistently undetectable EBV levels fell significantly with increasing time from transplant ($p < 0.0001$). Using the multivariate model, including time from transplant, no significant effect of age on DNAemia prevalence was detected (linear trend, OR = 0.89, CI = 0.75–1.04 per decade, $p = 0.16$), with no evidence for a nonlinear effect.

Analysis of factors associated with EBV infection

Univariate and multivariate analyses of demographic, treatment and infection in relation to EBV DNAemia at recruitment are shown in Table 4. EBV seronegativity at transplantation, a previous diagnosis of PTLD and non-White ethnicity were associated with greater rates of DNAemia. Those with EBV VCA positive EBNA positive antibody status at transplantation and those receiving MMF at recruitment had significantly lower rates of DNAemia ($p < 0.0001$) (Figure 1C).

Significantly lower lymphocyte counts were seen in individuals at recruitment on regimens including MMF (median 1.33×10^9/L, IQR = 0.95–1.70 vs. 1.46×10^9/L, IQR = 1.01–1.91, $p = 0.012$) and azathioprine (median 1.30×10^9/L, IQR = 0.87–1.73 vs. 1.46×10^9/L, IQR = 1.04–1.88, $p < 0.0001$) compared to those receiving other agents. Individuals on MMF were not significantly more lymphopenic than those on azathioprine. Individuals with a history of treatment for rejection including antithymocyte globulin (ATG) did not have significantly lower lymphocyte counts than others at recruitment.

Factors associated with HVL

Factors associated with HVL included: time of transplant recipient EBV seronegative status (11/49 vs. 20/355, OR = 4.9, CI 2.2–11.2, $p < 0.0001$), recipient VCA positive EBNA negative versus VCA positive EBNA positive serostatus (8/72 vs. 12/270, OR 2.7, CI 1.1–6.8, $p = 0.03$), current ciclosporin use (15/111 vs. 16/335, OR = 3.1, CI = 1.5–6.5, $p = 0.002$), monotherapy (13/102 vs. 18/344, OR = 2.6, CI = 1.2–5.6, $p = 0.009$) and VCA antibody levels ≥750 U/mL (22/31 vs. 68/233, OR = 5.9, CI 2.6–13.5,

Table 3: Recruitment Epstein–Barr virus (EBV) DNAemia status and subsequent DNAemia status at study end

EBV DNAemia (n = 446) at recruitment	N	EBV DNAemia at end of study	N (%)
Detectable	135	Detectable	67/135 (50)
Detectable log 3–3.9	83	Detectable	28/83 (34)
Detectable log 4–4.9	42	Detectable	30/42 (71)
Detectable log 5+	10	Detectable	9/10 (90)
Undetectable	311	Undetectable	290/311 (93)
Undetectable	311	Detectable	21/311 (7)

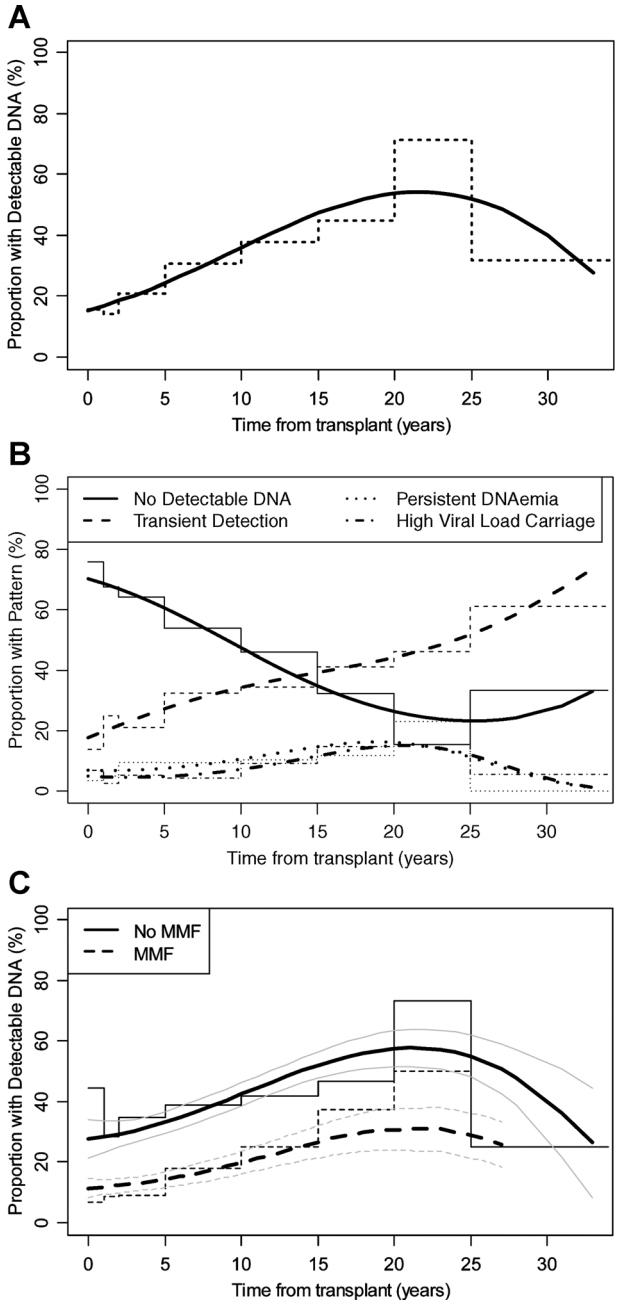

Figure 1: Proportions of patients versus time from transplant. (A) Detectable EBV DNA in the study population (stepped lines show mean values in 5-year intervals and the solid lines a cubic logistic regression fit to the data). (B) Patterns of EBV DNAemia during follow-up. (C) Proportion of patients with detectable EBV DNA treated with and without MMF (stepped lines show mean values in 5-year intervals and the bold lines a cubic logistic regression fit to the data). (C) 95% Confidence intervals (faint lines) are presented with the bold cubic logistic regression curves. EBV, Epstein–Barr virus; MMF, mycophenolate mofetil.

$p < 0.0001$). Time from transplant >10 years approached significance (18/187 vs. 13/259, OR = 2.0, CI = 1.0–4.2, $p = 0.059$). Low prevalence of high viral load detection was associated with basiliximab induction (8/204 vs. 23/244, OR = 0.4, CI = 0.2–0.9, $p = 0.022$), current MMF (2/132 vs. 29/264, OR = 0.12, CI = 0.03–0.5, $p = 0.0009$) and lymphopenia $<1 \times 10^9$/L at recruitment (1/99 vs. 30/346, OR = 0.1, CI = 0.01–0.8, $p = 0.008$).

Patient and graft survival

Three-year patient survival was not significantly different between the recruited and the nonrecruited group (hazard ratio [HR] = 1.14, CI = 0.60–2.13, $p = 0.68$) (Figure 2A) nor was survival different between individuals with detectable EBV at recruitment and those without (HR = 1.84, CI = 0.76–4.48, $p = 0.18$) (Figure 2B). Graft loss with subsequent return to dialysis occurred in 11 recruited patients during follow-up with no significant association with EBV detection at recruitment ($p = 0.10$). Kidney function (estimated GFR [eGFR]) declined significantly over the study period, -3.68 mL/min/1.73 m^2, $p < 0.0001$, but the rate of decline was not associated with EBV DNA detection status at recruitment, or the pattern of EBV DNAemia.

Incidence of PTLD

PTLD (first presentation) was diagnosed in six individuals during follow-up, at a median time of 482 days (IQR 203–700) from recruitment. Freedom from PTLD in those with detectable EBV at recruitment was 96.6% compared to 99.4% with no detectable EBV (HR = 5.6, CI = 0.97–31.6, $p = 0.05$) (Figure 2C), and not significantly different between the recruited and nonrecruited groups (HR = 3.79, CI = 0.85–16.91, $p = 0.08$) (Figure 2D).

Characteristics of PTLD cases are presented in Table 5. All PTLD cases were EBV seropositive at recruitment, although EBNA antibody deficiency was seen in 4/6 (67%) compared to 22% of individuals in the study population overall (OR = 6.0, CI 1.0–33.3, $p = 0.039$). Histology was negative for EBV in Patients 1 and 2 who had persistent EBV DNAemia during the sampling period, and one of whom also had undetectable EBV in blood at diagnosis.

On univariate analysis, freedom from PTLD during the study period was associated with time from transplant (97.3% ≥10 years vs. 99.3% ≤10 years, $p = 0.026$), persistent EBV DNAemia (92.4% vs. 99%, $p = 0.035$), detection of EBV at recruitment (96.6% vs. 99.4%, HR = 6.2, CI = 1.1–36.0, $p = 0.043$), previous *Pneumocystis jiroveci* infection (90% vs. 98.7%, $p = 0.015$), warts (93.5% vs. 100%, $p < 0.0001$), nonmelanoma skin cancer (94.6% vs. 99.2%, $p = 0.021$), gum hypertrophy (75.5% vs. 99.1%, $p < 0.0001$) and EBV EBNA antibody deficiency (96% vs. 99%, $p = 0.014$). The incidence of PTLD was not significantly different between recruited, 6 cases/1325 patient years and nonrecruited patients, 1 case/1143 patient years (rate ratio 5.2, 95% CI = 0.6–238.1, $p = 0.09$).

Table 4: Detection of EBV DNA at recruitment and associated factors: univariate analysis and multivariate adjusted analysis

	Detectable DNA, n (%)		Univariate analysis		Multivariate adjusted analysis[1]	
Variables	Variable present	Variable absent	OR[2] 95% CI	p	OR[2] 95% CI	p
Age (linear trend)					0.9 (0.8–1.0) (per decade)	0.16
Age >mean (51 years)	79/264 (30)	74/235 (32)	0.9 (0.6–1.4)	0.78	0.8 (0.5–1.2)	0.20
Male gender	88/310 (28)	65/189 (34)	0.8 (0.5–1.1)	0.19	0.8 (0.5–1.2)	0.31
Non-White ethnicity	15/34 (44)	138/465 (30)	1.9 (0.9–4.0)	0.085	2.7 (1.2–5.8)	**0.014**
Living donor tranplant	26/107 (24)	127/392 (32)	0.7 (0.4–1.1)	0.11	0.9 (0.5–1.6)	0.71
Nondiabetic	123/441 (34)	19/58 (20)	2.0 (1.2–3.5)	**0.014**	1.5 (0.8–2.8)	0.17
Pretransplant EBV recipient serostatus						
VCA neg EBNA neg (seronegative)	27/54 (50)	110/397 (28)	2.6 (1.5–4.7)	**0.001**	1.9 (1.0–3.7)	**0.045**
VCA neg EBNA pos	5/16 (31)	132/435 (30)	1.0 (0.4–3.1)	0.94	0.9 (0.3–3.0)	0.92
VCA pos EBNA pos	78/297 (26)	59/154 (38)	0.6 (0.4–0.9)	**0.009**	0.6 (0.4–1.0)	**0.044**
VCA pos EBNA neg	27/84 (32)	110/367 (30)	1.1 (0.7–1.8)	0.70	1.3 (0.7–2.2)	0.42
Pretransplant EBV serostatus missing	16/48 (33)	137/451 (30)	1.1 (0.6–2.2)	0.67	0.8 (0.4–1.6)	0.52
Transplant no. >1	31/94 (33)	122/404 (30)	1.1 (0.7–1.8)	0.62	1.2 (0.7–1.9)	0.60
Treated rejection	40/105 (38)	113/394 (29)	1.5 (1–2.4)	0.074	1.4 (0.8–2.2)	0.21
Anti-thymocyte globulin	11/32 (34)	142/467 (30)	1.2 (0.6–2.6)	0.693	1.4 (0.6–3.3)	0.42
ECOG ≥2	30/89 (34)	120/410 (30)	1.2 (0.7–1.9)	0.525	1.1 (0.7–1.9)	0.66
Karnofsky <80	34/95 (36)	116/404 (29)	1.3 (0.8–2.2)	0.218	1.4 (0.8–2.3)	0.25
Basiliximab induction	41/225 (18)	108/274 (40)	0.33 (0.2–0.5)	<**0.0001**	0.8 (0.4–1.7)	0.59
Monotherapy	45/109 (41)	107/390 (28)	1.9 (1.2–2.9)	**0.0095**	0.9 (0.6–1.6)	0.80
Dual therapy (vs. triple)	87/276 (32)	20/110 (18)	2.1 (1.2–3.6)	**0.012**	1.2 (0.6–2.1)	0.62
Triple therapy	20/110 (18)	132/389 (34)	0.4 (0.3–0.7)	**0.0015**	0.9 (0.5–1.7)	0.70
Current tacrolimus	67/278 (24)	86/221 (39)	0.5 (0.3–0.7)	**0.0004**	1.0 (0.5–1.9)	0.91
Current ciclosporin	54/127 (42)	99/372 (27)	2.0 (1.3–3.1)	**0.0012**	1.0 (0.5–2.0)	0.90
Current MMF	31/205 (15)	122/294 (41)	0.3 (0.2–0.4)	<**0.0001**	0.3 (0.2–0.6)	<**0.0001**
Current azathioprine	41/110 (37)	112/389 (29)	1.5 (0.9–2.3)	0.101	0.8 (0.4–1.4)	0.39
Current prednisolone	85/265 (32)	68/234 (29)	1.2 (0.8–1.7)	0.497	1.3 (0.8–2.3)	0.33
Current sirolimus	3/12 (25)	150/487 (31)	0.7 (0.2–2.8)	0.999	0.6 (0.1–2.6)	0.49
PTLD diagnosis	15/22 (68)	134/477 (28)	5.3 (2.3–12.0)	<**0.0001**	3.4 (1.4–8.1)	**0.006**
CKD 4 or 5	37/100 (30)	113/399 (28)	1.0 (0.6–1.5)	0.999	1.2 (0.7–2.0)	0.54
CKD 1–2	31/105 (32)	119/394 (31)	1.0 (0.7–1.7)	0.903	1.4 (0.8–2.3)	0.25
Hemoglobin < 11.5	44/121 (36)	106/378 (20)	1.4 (0.9–2.4)	0.174	1.3 (0.8–2.0)	0.37
Leucopenia	9/24 (38)	141/490 (31)	1.3 (0.6–3.1)	0.503	1.2 (0.5–2.0)	0.67
Raised ALT	4/29 (14)	146/458 (32)	0.3 (0.1–1.0)	**0.04**	0.5 (0.2–1.6)	0.23
Thrombocytopenia	14/42 (33)	136/438 (31)	1.1 (0.6–2.2)	0.73	1.2 (0.6–2.6)	0.66
Lymphopenia $< 1.5 \times 10^9$	80/290 (28)	73/209 (35)	0.7 (0.5–1.0)	0.094	0.8 (0.5–1.3)	0.37
Lymphopenia $< 1 \times 10^9$	25/110 (23)	128/389 (33)	0.6 (0.4–1.0)	**0.046**	0.6 (0.4–1.1)	0.077
Lymphocytosis	1/3 (33)	152/496 (31)	1.1 (0.02–21.9)	0.99	1.4 (0.1–16.2)	0.78
Neutropenia	6/22 (27)	147/477 (31)	0.8 (0.3–2.2)	0.82	0.8 (0.3–2.4)	0.72
PJP infection	4/10 (40)	149/489 (30)	1.5 (0.5–5.4)	0.50	1.8 (0.5–7.4)	0.40
Treated CMV disease	18/68 (26)	135/431 (31)	0.8 (0.4–1.4)	0.48	1.0 (0.6–1.9)	0.93
Nonmelanoma skin cancer	32/76 (42)	121/423 (29)	1.8 (1.1–3.0)	**0.03**	1.5 (0.9–2.6)	0.15
Warts	45/115 (39)	108/384 (28)	1.6 (1.1–2.5)	**0.029**	1.1 (0.7–1.8)	0.73
Herpes simplex virus oral	34/113 (30)	119/386 (31)	1.0 (0.6–1.5)	0.908	0.9 (0.5–1.4)	0.58
Shingles	28/73 (38)	125/426 (29)	1.5 (0.9–2.5)	0.132	1.8 (1.0–3.2)	0.059
Primary varicella	5/13 (39)	148/486 (31)	1.4 (0.5–4.4)	0.550	1.0 (0.3–3.6)	0.97
Pulmonary tuberculosis	2/4 (50)	151/495 (30)	2.3 (0.3–16.3)	0.590	3.4 (0.4–28.6)	0.26
Extra-pulmonary tuberculosis	3/3 (100)	150/496 (30)	(0–Inf)	**0.028**	(0–Inf)	0.97
BK viremia	5/19 (26)	148/480 (31)	0.8 (0.3–2.3)	0.803	1.4 (0.5–4.4)	0.53

ALT, alanine transaminase; CI, confidence interval; CKD, chronic kidney disease; CMV, cytomegalovirus; EBNA, Epstein–Barr nuclear antigen-1; EBV, Epstein–Barr virus; ECOG, Eastern Cooperative Oncology Group; MMF, mycophenolate mofetil; OR, odds ratio; PJP, Pneumocystis jiroveci infection; PTLD, posttransplant lymphoproliferative disease; VCA, viral capsid antigen.

[1]Cubic model adjusting for time from transplant, gender, ethnicity, previous PTLD diagnosis, immunosuppressive therapies including monotherapy, current tacrolimus, current ciclosporin and current MMF use.

[2]ORs shown to one significant figure in the table.

Values with $p < 0.05$ are highlighted in bold.

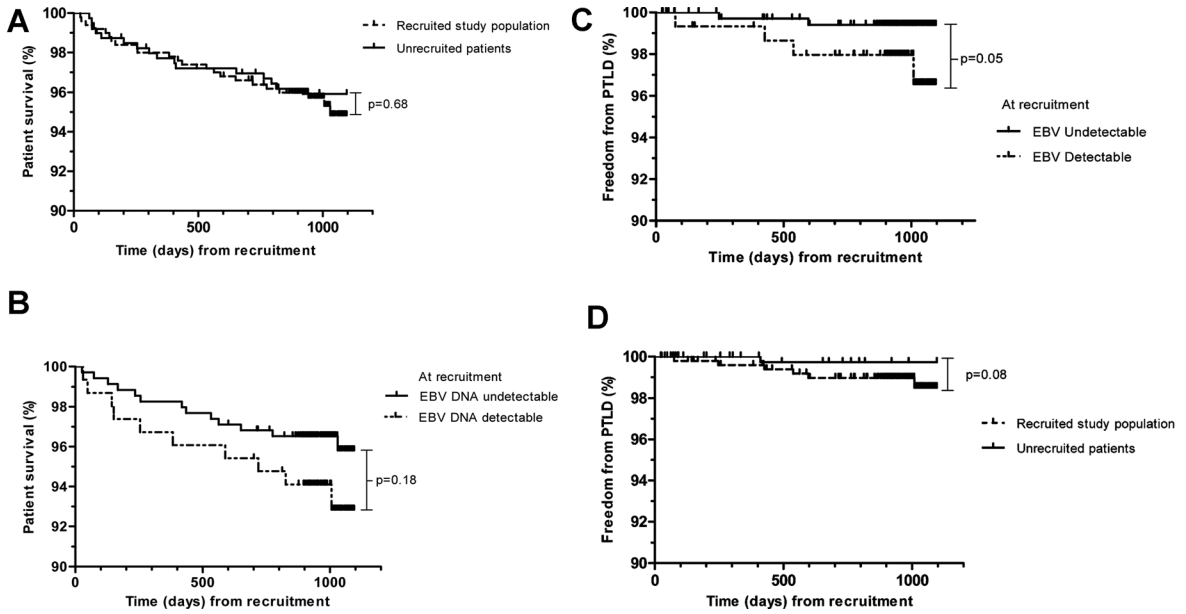

Figure 2: (A) Overall patient survival during the study follow-up, recruited study population versus unrecruited transplant recipients under follow-up, HR = 1.14, CI = 0.60–2.13, p = 0.68. (B) Overall patient survival during the study follow-up, undetectable EBV DNAemia versus detectable EBV DNAemia at recruitment, HR = 1.84, CI = 0.76–4.48, p = 0.18. (C) Freedom from PTLD during the study follow-up, undetectable EBV DNAemia versus detectable EBV DNAemia at recruitment (HR = 5.6, CI = 0.97–31.6, p = 0.05). (D) Freedom from PTLD during the study follow-up, recruited study population versus nonrecruited transplant recipients under follow-up (HR = 3.79, CI = 0.85–16.91, p = 0.08). Kaplan–Meier survival curve and log-rank test, censored for graft loss and death. CI, confidence interval; EBV, Epstein–Barr virus; HR, hazard ratio; PTLD, posttransplant lymphoproliferative disease.

A previous history of PTLD (65% EBV tissue positive) was recorded in 22 individuals at recruitment of whom 15/22 (68%) had EBV DNAemia. Patterns of EBV DNAemia during the study in 17/22 patients with ≥3 samples included NDNA in 6/17 (35%) (including 4/6 with a history of EBV negative PTLD), PDNA in 5/17 (29%) (all EBV tissue positive disease) and 2 HVL (both EBV positive disease, 1 polymorphic, 1 infectious mononucleosis early lesion). Relapse of PTLD was seen in three individuals, including a late relapse 6.4 years after diagnosis in one patient with a history of EBV negative diffuse large B cell lymphoma. The recurrent disease was also EBV negative diffuse large B cell lymphoma with no detectable EBV DNA in blood. Compared to nonrecruited patients, study patients had a significantly greater proportion with a history of PTLD, 3/395 (0.8%) non-recruited versus 22/499 (4.4%) recruited (OR = 6.0, 95% CI = 1.8–20.3, p = 0.0008).

Table 5: Characteristics of PTLD cases diagnosed during the study period

Patient no.	Age	Time from transplant (years)	EBV serostatus pre-Tx[1]	EBV VL[2] recruitment	EBV DNAemia pattern	PTLD WHO class	PTLD EBV[3] status	EBV VL[4] PTLD
1	58	3	Unknown	3.4	PDNA	DLBCL	−	<3.0
2	74	23	Positive	4.3	PDNA	Mono	−	3.9
3	66	19	Positive	<3.0	TDNA	DLBCL	+	3.0
4	37	16	Negative	3.8	HVL	HL	+	3.7
5	67	15	Negative	<3.0	NDNA	Poly	−	<3.0
6	59	20	Positive	5.3	TDNA	DLBCL	+	3.4

DLBCL, diffuse large B-cell lymphoma; EBER, Epstein–Barr encoding region; EBV, Epstein–Barr virus; HL, Hodgkin's lymphoma; HVL, high viral load carriage; Mono, monomorphic; NDNA, no detectable DNA; PDNA, persistent DNAemia; Poly, polymorphic; PTLD, posttransplant lymphoproliferative disease; TDNA, transient detection; Tx, transplant; VL, viral load, WHO, World Health Organization.
[1]EBV recipient pretransplant serostatus.
[2]EBV VL at study recruitment $\log_{10}$ copies/mL.
[3]EBV status of PTLD tissue at histological examination (*in situ* hybridization for EBER); − not detected, + EBER detected.
[4]EBV VL at time of PTLD diagnosis $\log_{10}$ copies/mL.

Discussion

Clinical guidelines and recent studies of EBV infection focus predominantly on incidence and implications of viremia in the first posttransplant year (3). Screening for EBV DNA during the first year is recommended, particularly for high-risk EBV donor seropositive recipient seronegative patients. There is little reporting on prevalence, patterns and outcomes of EBV infection beyond the first year. Such information is important because most PTLD in this population presents late, beyond the first year, and over 50% of late cases are associated with EBV infection (8,9). In addition, there are conflicting analyses relating to the associations of EBV DNAemia with PTLD development, graft dysfunction and loss, acute rejection, opportunistic infection, adverse events, risk of death and overall burden of immunosuppression (6,7,12,14–17).

Bamoulid et al, Holman et al and Holmes et al reported EBV DNAemia rates from 40% to 56% in adult renal transplant recipients during the first posttransplant year (6,13,18). We report a lower rate of EBV DNAemia (>1000 copies/mL) at recruitment of 16% and 24% during follow-up in individuals within the first posttransplant year, while 31% of the study population at recruitment and 46% of individuals overall, had one or more samples with detectable DNA during the study year. However, Bamoulid et al (6) reported a similar prevalence (19%) of DNAemia levels >1000 copies/mL. Further, Holmes et al (18) reported 7% DNAemia >10 000 copies/mL, which compares to our prevalence of 7% chronic HVL (>10 000 copies/mL) in the first year posttransplant.

In this cross-sectional analysis, DNAemia prevalence and persistence appear to increase, rather than fall, with time from transplant, perhaps as a consequence of prolonged exposure to immunosuppression and/or immunosenescence, although we could not confirm an effect of age on DNAemia in analyses adjusted for time. However, studies such as this may suffer from selection effects, with prevalence rates being conditional on survival. This is a limitation of our study and a cohort study would be required to further investigate factors associated with late EBV DNAemia from time of transplant. Attention should be given to co-morbidities such as history of diabetes, cardiovascular disease and autoimmune disease, while analysis of markers of under-immunosuppression such as donor-specific antibody detection, proteinuria and rate of graft decline as well as over-immunosuppression such as viral, bacterial infection or skin cancer may also be of interest.

High DNAemia rates during the first year might be expected as immunosuppression levels are high and seronegative recipients experience primary infection. Falling rates of detection with increasing time from transplant have been reported in heart and lung recipients (19). In our series, low rates in the first year may be due to sample size, but may reflect immunosuppressive practice, including MMF and minimal use of T cell depleting antibody.

In analyses adjusted for time from transplant, MMF was the only agent with a significant association with DNAemia rates. Reduced incidence of DNAemia in those receiving MMF has been noted in other studies (6,18,20). It has been suggested that this may be due to an anti-B cell effect, reducing the EBV-carrying B lymphocyte population (18). Treatment with rituximab is associated with transient depletion of B cell numbers with corresponding falls in EBV viral loads, and studies have also reported subsequent rises in viral loads as B cell numbers are later reconstituted (6,21,22). Individuals on MMF or azathioprine in our study had lower total lymphocyte counts than those not on anti-metabolite/proliferatives, but only MMF was significantly associated with DNAemia. Analysis of B cell numbers in relation to EBV viral loads in larger numbers of patients on MMF may be helpful. These observations raise the following questions. Does the observed lower risk of EBV DNAemia in MMF-treated patients correspond to a reduced risk of EBV positive PTLD? Should we consider changing asymptomatic individuals with high-level EBV DNAemia to MMF, and should low-dose MMF with steroids be the maintenance agents of choice in new cases of EBV positive PTLD, rather than low-dose calcineurin inhibitors? PTLD risk registry analyses suggest that MMF-treated individuals overall may have a reduced incidence and risk of PTLD development (23–26), although recent analyses report a high prevalence of MMF use in primary central nervous system PTLD (27,28). To date there is no clear evidence to guide immunosuppression prescribing in the late posttransplant period as treatment or prevention of EBV-related disease or PTLD.

In the event of detection of EBV DNAemia in stable patients, the likelihood of persistence of DNAemia over at least the following year of follow-up was increased if the recipient was EBV seronegative at transplant (6,14,18), and the higher the viral load at recruitment with 88% of those with initially undetectable levels remaining free of EBV detection.

The implications of EBV DNAemia in otherwise stable transplant recipients in the late posttransplant period are unclear. EBV is a latent herpes virus, and after primary infection, it is anticipated that a steady state will be reached, where numbers of EBV-infected B cells are controlled by effective EBV-specific T cell responses (29). EBV DNA in blood is found in healthy members of the general population, although typically at significantly lower levels than following transplantation (18,30). While detection of CMV and BK virus DNA in blood, particularly if >1000 copies/mL, is typically associated with clinical symptoms and end-organ damage, detection of late EBV DNAemia may not identify those with a clinical illness requiring treatment, and/or those who are over-immunosuppressed. In our study those with DNAemia did not have significantly

poorer survival during follow-up or poorer graft-related outcomes, nor did they have greater rates of specific symptoms, hematological or biochemical abnormalities. Further, increased DNAemia prevalence was not associated with previous ATG use, higher calcineurin inhibitor trough concentration, or greater numbers of maintenance immunosuppressive agents. In support of an ''over-immunosuppressed'' argument, we show, in univariate analysis, an association of DNAemia with a history of nonmelanoma skin cancer, warts, extra-pulmonary tuberculosis (albeit small numbers), time from transplant and longer duration of immunosuppression.

Recent studies have reported associations between EBV DNAemia in the first year after transplant and opportunistic infections, adverse events, and in some studies, greater rates of graft loss and graft dysfunction (6,14,20,31,32). In the Bamoulid et al report, there is also concern raised that preemptive reduction of immunosuppression for EBV DNAemia persistently $>10^4$ log/mL copies may influence subsequent graft loss (6).

Risk of PTLD has been associated with EBV donor positive/recipient negative individuals, those with higher levels of EBV DNA detection, chronic HVL, detectable DNA in plasma and those receiving T cell depleting antibodies (7,8,23,33–36). Preemptive strategies to reduce PTLD incidence include reduction of immunosuppression, and more recently, the use of rituximab in transplant patients with persistent high-level DNAemia. Reduction in incidence of PTLD is reported in liver transplant and pediatric populations with such strategies (5–7,37–42). We calculated an increased risk of PTLD during follow-up for those with DNAemia at recruitment compared to those without detectable DNAemia. Should screening for EBV DNA be performed in the late posttransplant period, how frequently and in whom should immunosuppression be reduced preemptively? We show late EBV DNAemia is common, 46% $\geq$1 sample, and 16% with persistent viral loads $>$1000 copies/mL, but is not associated with poorer graft function or specific symptoms. However, it is of note that two of six PTLD cases (33%) occurred in seronegative recipients (high risk). Further, an EBV tissue histology-negative patient had persistent EBV DNAemia during the sampling period, yet, at the time of development of PTLD, had undetectable EBV DNA in blood. EBV seronegative recipients also had a greater incidence of DNAemia at recruitment and of chronic HVL. While only 10% of the study population, they accounted for at least 33% of PTLD cases during the follow-up period.

Screening EBV seronegative recipients for DNAemia, symptoms and lymphadenopathy for the lifetime of their graft is logistically and clinically sensible. This is a small group with a greater risk of PTLD than seropositive recipients, persisting into the late posttransplant period. However, there is no evidence from our study to support the use of EBV DNAemia detection in the low-risk seropositive group to prompt a change of immunosuppression or investigation for PTLD. Attention should perhaps focus as much on screening for symptoms and regular clinical examinations as on EBV DNAemia screening, particularly as EBV negative histology PTLD accounts for up to 50% of late PTLD cases in adult renal transplant recipients.

Acknowledgments

We are grateful to Dr. Susan Martin and Dr. Judith Worthington from the regional tissue typing laboratory; to Alan Lord and Ben Brown in the regional Health Protection Laboratory; to Victoria Bowman and Kate Atkinson's; support with the transplant and EBV study databases at MRI; to Will Hulme and Michael Patrick at NHS Blood and Transplant; and to Sister Lisa Laycock and the transplant outpatient team at Manchester Royal. Special thanks go to our transplant patients who willingly took part in the study. MM was in receipt of an Astellas Renal Transplant Clinical Fellowship. MLP was in receipt of an unrestricted educational grant from Amgen. Funding support for the study was provided by Central Manchester University Hospital Foundation Trust Biomedical Research Centre in the form of research grants awarded to the team in open competition.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

Author Contributions

Each author's specific contributions to the work are as follows: MM, MLP, PEK, PJV, SAR and BC participated in the research design; MM, BC, MLP, SAR, PEK and PJV participated in the writing of the paper; MM, SLJ, PEK and BC participated in the performance of the research; MM and SAR participated in data analyses and all authors approved the final manuscript.

References

1. Parker A, Bowles K, Bradley J, et al. Diagnosis of post-transplant lymphoproliferative disorder in solid organ transplant recipients—BCSH and BTS Guidelines. Br J Haematol 2010; 149: 675–692.
2. 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: 31–33, 35–36.
3. KDIGO. KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant 2009; 9 (Suppl 3): S1–S155.
4. Green M, Michaels M. Epstein–Barr virus infection and posttransplant lymphoproliferative disorder. Am J Transplant 2013; 13 (Suppl 3): S41–S54.

5. Martin S, Dodson B, Wheeler C, Davis J, Pesavento T, Bumgardner G. Monitoring infection with Epstein–Barr virus among seromismatch adult renal transplant recipients. Am J Transplant 2011; 11: 1058–1063.
6. Bamoulid J, Courivaud C, Coaquette A, et al. Subclinical Epstein–Barr virus viremia among adult renal transplant recipients: Incidence and consequences. Am J Transplant 2013; 13: 656–662.
7. Lee T, Savoldo B, Rooney C, et al. Quantitative EBV viral loads and immunosuppression alterations can decrease PTLD incidence in pediatric liver transplant recipients. Am J Transplant 2005; 5: 2222–2228.
8. Morton M, Coupes B, Roberts S, et al. Epidemiology of posttransplantation lymphoproliferative disorder in adult renal transplant recipients. Transplantation 2013; 95: 470–478.
9. Caillard S, Lamy F, Quelen C, et al. Epidemiology of posttransplant lymphoproliferative disorders in adult kidney and kidney pancreas recipients: Report of the French registry and analysis of subgroups of lymphomas. Am J Transplant 2012; 12: 682–693.
10. Niesters HGM, Van Esser J, Fries E, Wolthers KC, Cornelissen J, Osterhaus ADME. Development of a real-time quantitative assay for detection of Epstein–Barr virus. J Clin Microbiol 2000; 38: 712–715.
11. Allen U, Preiksaitis J. Epstein–Barr virus and posttransplant lymphoproliferative disorder in solid organ transplantation. Am J Transplant 2013; 6: 107–120.
12. Bingler MA, Feingold B, Miller SA, et al. Chronic high Epstein–Barr viral load state and risk for late-onset posttransplant lymphoproliferative disease/lymphoma in children. Am J Transplant 2008; 8: 442–445.
13. Holman CJ, Karger AB, Mullan BD, Brundage RC, Balfour HH. Quantitative Epstein–Barr virus shedding and its correlation with the risk of post-transplant lymphoproliferative disorder. Clin Transplant 2012; 26: 741–747.
14. San-Juan R, De Dios B, Navarro D, et al. Epstein–Barr virus DNAemia is an early surrogate marker of the net state of immunosuppression in solid organ transplant recipients. Transplantation 2012; 95: 688–693.
15. 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.
16. Tsai DE, Nearey M, Hardy CL, et al. Use of EBV PCR for the diagnosis and monitoring of post-transplant lymphoproliferative disorder in adult solid organ transplant patients. Am J Transplant 2002; 2: 946–954.
17. Green M, Soltys K, Rowe DT, Webber SA, Mazareigos G. Chronic high Epstein–Barr viral load carriage in pediatric liver transplant recipients. Pediatr Transplant 2009; 13: 319–323.
18. Holmes M, 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.
19. Haque T, Thomas JA, Parratt R, Hunt BJ, Yacoub MH, Crawford DH. A prospective study in heart and lung transplant recipients correlating persistent Epstein–Barr virus infection with clinical events. Transplantation 1997; 64: 1028–1034.
20. Li L, Chaudhuri A, Weintraub LA, et al. Subclinical cytomegalovirus and Epstein–Barr virus viremia are associated with adverse outcomes in pediatric renal transplantation. Pediatr Transplant 2007; 11: 187–195.
21. Van Esser JW, Niesters HG, Van der Holt B, et al. Prevention of Epstein–Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. Blood 2002; 99: 4364–4369.
22. Savoldo B, Rooney CM, Quiros-Tejeira RE, et al. Cellular immunity to Epstein–Barr virus in liver transplant recipients treated with rituximab for post-transplant lymphoproliferative disease. Am J Transplant 2005; 5: 566–572.
23. Sampaio M, Cho Y, Shah T, Bunnapradist S, Hutchinson I. Association of immunosuppressive maintenance regimens with posttransplant lymphoproliferative disorder in kidney transplant recipients. Transplantation 2012; 93: 73–81.
24. 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.
25. Birkeland SA, Hamilton-Dutoit S. Is posttransplant lymphoproliferative disorder (PTLD) caused by any specific immunosuppressive drug or by the transplantation per se? Transplantation 2003; 76: 984–988.
26. Funch DP, Ko HH, Travasso J, et al. Posttransplant lymphoproliferative disorder among renal transplant patients in relation to the use of mycophenolate mofetil. Transplantation 2005; 80: 1174–1180.
27. Evens AM, Choquet S, Kroll-Desrosiers AR, et al. Primary CNS posttransplant lymphoproliferative disease (PTLD): An international report of 84 cases in the modern era. Am J Transplant 2013; 13: 1512–1522.
28. Boersma MN, Van der Zanden A, Laverman GD, Sanders JS, De Vries PAM. Epstein–Barr virus-positive post-transplant lymphoproliferative disorder of the central nervous system, after renal transplantation with a discrepancy in viral load between peripheral blood and cerebrospinal fluid. Transplant Int 2012; 25: e113–e116.
29. Rickinson A, Kieff E. Epstein–Barr virus. In: Snipe DM, Howley PM, eds. *Field's virology*. Lippincott, Williams & Wilkins, 2006, pp. 2657–2701.
30. Njie R, Bell A, Jia H, et al. The effects of acute malaria on Epstein–Barr virus (EBV) load and EBV-specific T cell immunity in Gambian children. J Infect Dis 2009; 199: 31–38.
31. Ahya VN, Douglas LP, Andreadis C, et al. Association between elevated whole blood Epstein–Barr virus (EBV)-encoded RNA EBV polymerase chain reaction and reduced incidence of acute lung allograft rejection. J Heart Lung Transplant 2007; 26: 839–844.
32. Smith JM, Corey L, Bittner R, et al. Subclinical viremia increases risk for chronic allograft injury in pediatric renal transplantation. J Am Soc Nephrol 2010; 21: 1579–1586.
33. Tsai D, Douglas L, Andreadis C, et al. EBV PCR in the diagnosis and monitoring of posttransplant lymphoproliferative disorder: Results of a two-arm prospective trial. Am J Transplant 2008; 8: 1016–1024.
34. Opelz G, Daniel V, Naujokat C, Dohler B. Epidemiology of pretransplant EBV and CMV serostatus in relation to posttransplant non-Hodgkin lymphoma. Transplantation 2009; 88: 962–967.
35. Sampaio M, Cho Y, Shah T, Bunnapradist S, Hutchinson I. 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.
36. Van Leeuwen MT, Grulich AE, Webster AC, et al. Immunosuppression and other risk factors for early and late non-Hodgkin lymphoma after kidney transplantation. Blood 2009; 114: 630–637.
37. McDiarmid SV, Jordan S, Kim GS, et al. Prevention and preemptive therapy of postransplant lymphoproliferative disease in pediatric liver recipients. Transplantation 1998; 66: 1604–1611.
38. Tanaka E, Sato T, Ishihara M, et al. Asymptomatic high Epstein–Barr viral load carriage in pediatric renal transplant recipients. Pediatr Transplant 2011; 15: 306–313.

39. 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.
40. Blaes AH, Cao Q, Wagner JE, Jo-Anne HY, Weisdorf DJ, Brunstein CG. Monitoring and preemptive rituximab therapy for Epstein–Barr virus reactivation after anti-thymocyte globulin containing nonmyeloablative conditioning for umbilical cord blood transplantation. Biol Blood Marrow Transplant 2010; 16: 287–291.
41. Wagner HJ, Wessel M, Jabs W, et al. Patients at risk for development of posttransplant lymphoproliferative disorder: Plasma versus peripheral blood mononuclear cells as material for quantification of Epstein–Barr viral load by using real-time quantitative polymerase chain reaction. Transplantation 2001; 72: 1012–1019.
42. Tsai D, Hardy C, Tomaszewski J, et al. Reduction in immunosuppression as initial therapy for posttransplant lymphoproliferative disorder: Analysis of prognostic variables and long-term follow-up of 42 adult patients. Transplantation 2001; 71: 1076–1088.