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Ruijter, B.N.; Wolterbeek, R.; Hew, M.; Reeven, M. van; Helm, D. van der; Dubbeld, J.; ... ; Hoek, B. van

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Epstein–Barr Viral Load Monitoring Strategy and the Risk for Posttransplant Lymphoproliferative Disease in Adult Liver Transplantation

A Cohort Study

Bastian N. Ruijter, MD; Ron Wolterbeek, MD, MSc; Mitchell Hew, MD; Marjolein van Reeven, MD;
Danny van der Helm, MSc, PhD; Jeroen Dubbeld, MD; Maarten E. Tushuizen, MD, PhD; Herold Metselaar, MD, PhD;
Ann C.T.M. Vossen, MD, PhD; and Bart van Hoek, MD, PhD

Background: Primary infection with or reactivation of Epstein–Barr virus (EBV) can occur after liver transplant (LT) and can lead to posttransplant lymphoproliferative disease (PTLD). In pediatric LT, an EBV-DNA viral load (EBV VL) monitoring strategy, including the reduction of immunosuppression, has led to a lower incidence of PTLD. For adult LT recipients with less primary infection and more EBV reactivation, it is unknown whether this strategy is effective.

Objective: To examine the effect of an EBV VL monitoring strategy on the incidence of PTLD after LT in adults.

Design: Cohort study.

Setting: Two university medical centers in the Netherlands.

Patients: Adult recipients of first LT in Leiden between September 2003 and January 2017 with an EBV VL monitoring strategy formed the monitoring group (M1), recipients of first LT in Rotterdam between January 2003 and January 2017 without such a strategy formed the contemporary control group (C1), and those who had transplants in Leiden between September 1992 and September 2003 or Rotterdam between 1986 and January 2003 formed the historical control groups (M0 and C0, respectively).

Measurements: Influence of EBV VL monitoring on incidence of PTLD.

Results: After inverse probability of treatment weighting of the 4 groups to achieve a balance among the groups for important patient characteristics, differences within hospitals between the historical and recent era in cumulative incidences—expressed as the number of events per 1000 patients measured at 5-, 10-, and 15-year follow-up—showed fewer events in the contemporary era in both centers. This difference was considerably larger in the monitoring center, whereas the 95% CI included the null value of 0 for point estimates.

Limitation: Retrospective, low statistical power, and incompletely balanced groups, and non-EBV PTLD cannot be prevented.

Conclusion: Monitoring EBV VL may reduce PTLD incidence after LT in adults; larger studies are warranted.

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P rimary infection or reactivation of Epstein–Barr virus (EBV) after transplant can lead to posttransplant lymphoproliferative disease (PTLD) (1–3). The reported cumulative incidence of PTLD after liver transplant (LT) was 2.9% in adults and 9.7% in children with 3 to 20 years of follow-up (1981 to 1998) (4) and 2.8% in mainly adults (1989 to 2010) (5). After LT in 2283 children, the incidence decreased from 4.2% between 1995 and 2001 to 1.7% between 2002 and 2007 (6). A registry including 15 631 LTs (1985 to 2001) reported a relative risk for lymphoma of 24.6 in the first year and 7.3 to 11.2 per year during the following 9 years compared with the general population; the increased relative risk decreased with increasing recipient age at LT (7). This difference between adults and children is mainly related to the higher incidence of primary EBV infection after LT in children (1–3, 6, 8–11). In children and adolescents, the risk

factors for PTLD are EBV seronegativity, immunosuppression intensity, and first year after transplant (6, 8–11). In adult recipients, PTLD can occur later after LT, and risk factors are less well known (1–3, 10). Epstein–Barr virus viral load (EBV VL) is associated with PTLD and symptomatic EBV, although most cases remain asymptomatic (12, 13). Epstein–Barr virus–negative PTLD can occur, usually longer after transplant and at an older age (1–3, 10). A monitoring strategy of repeated EBV VL measurement with reduction of immunosuppression for a detectable load after pediatric LT led to lower cumulative incidences of PTLD than in the literature (11, 14–22). In addition, after pediatric LT, such a monitoring strategy also resulted in a lower incidence of PTLD than in historical control groups without monitoring from the same center: 2% (1994 to 2002) versus 16% (2003 to 2007) (23), 5% versus 10% (24), 2% post-2001 versus 16% pre-2001 (25), and 5.7% versus 10.2% (26). There have been no studies with contemporary control groups. Although some studies have suggested that monitoring of EBV VL after LT in adults would have limited value (27), this is unknown. This study aimed to assess the value of an EBV VL monitoring

strategy with immunosuppression reduction after LT in adult recipients, with adjustment for key confounders.

METHODS

Study Population

In this retrospective cohort study, all deceased donors with first orthotopic LT from 2 LT centers in Leiden (center M) and Rotterdam (center C), the Netherlands, were included. Patients with auxiliary LT or a follow-up less than 2 weeks were excluded. Patients from Leiden with the EBV VL monitoring strategy and a transplant between September 2003 and January 2017 formed the EBV VL monitoring group (M1). Patients who had a LT in Rotterdam between January 2003 and January 2017 without EBV monitoring formed the contemporary control group (C1). This allowed for a sufficient follow-up. Two historical control groups with a first LT without EBV VL monitoring were formed by 2 historical cohorts: from September 1992 to September 2003 in Leiden (M0) and from 1986 to January 2003 in Rotterdam (C0). Follow-up was done until January 2020, death, or loss to follow-up. In cases of retransplant, follow-up was continued. Demographic characteristics and clinical features were retrieved from transplant registry databases, patient files, and electronic patient charts. The EBV serostatus of donors was unknown given that this was not part of donor screening in the Eurotransplant region. Immunosuppression was similar in the 2 centers, both in the 2 contemporary cohorts and the 2 historical cohorts. Changes in immunosuppression between historical and contemporary cohorts were also similar in the 2 centers, as shown in the Supplement (available at Annals.org).

Baseline characteristics examined were age, sex, underlying liver disease, IgG anti-EBV status, and initial immunosuppression.

Monitoring of EBV VL and Preemptive Strategy

For assessment of EBV VL, DNA was isolated from EDTA plasma using the MagNA Pure LC Total Nucleic Acid Isolation Kit and a MagNA Pure LC Instrument (Roche Diagnostics). Real-time quantitative polymerase chain reaction testing for EBV DNA was done as previously described (1 copy/mL = 1 IU/mL) on an iCycler iQ Multi-Color Real Time PCR Detection System (Bio-Rad) (28). The EBV VL detection limit was 100 IU/mL. From September 2003, all consecutive LT recipients in Leiden had weekly EBV-DNA monitoring during the first month, biweekly monitoring in the second month, and then monthly or at additional visits until 1 year after LT. After the first year, the EBV-DNA load was measured at least yearly and frequently more often. A detectable EBV VL should be followed by another VL measurement within 2 months. According to the protocol, during the first year after LT in case of 2 measurable EBV VLs within 2 months, immunosuppression should be reduced by dose reduction of the calcineurin inhibitor and/or dose reduction or cessation of mycophenolate mofetil, azathioprine, or prednisolone if possible. If EBV VL did not decrease, further reduction of immunosuppression was required, and

with further persistence of EBV VL positivity, 1 dose of 375 mg/m^2 intravenous anti-CD20 (rituximab, Roche) was administered while temporarily continuing immunosuppression with only low-dose prednisolone. Physicians were also allowed to lower immunosuppression at the first detectable EBV-DNA load or after the first year—at 1 or more detectable EBV VL. If the liver enzymes remained stable, the lower immunosuppression was continued long term; otherwise, immunosuppression slowly increased after at least 2 negative EBV VLs.

Diagnosis of PTLD

According to the World Health Organization's classification in 2016, PTLD is a lymphocyte or plasmatic proliferation arising in a recipient of solid organ or bone marrow allogeneic transplant with enlarged lymph nodes and/or organ involvement. Posttransplant lymphoproliferative disease can be early benign, polyclonal polymorphic, or monomorphic (M-PTLD), often monoclonal and fulfilling criteria of non-Hodgkin lymphoma–type or classic Hodgkin lymphoma–type PTLD (29). These different types of PTLD are considered different stages in the process of malignant transformation and can coexist even in the same tissue, making subclassification difficult (1, 2). All PTLD cases were included in the analysis. Staging was done according to the Ann Arbor system: a positron emission tomography–computed tomography scan (in early years, computed tomography of chest and abdomen), and if no positron emission tomography–computed tomography scan was done, a bone marrow biopsy was usually done; in some cases, peripheral blood flow cytometry was done, and histopathology from suspicious lymph nodes or involved organs was always obtained. Treatment before 1999 was done as described previously (30), and treatment after 1999 was done according to current standards (31, 32).

Outcomes

Cumulative incidence of PTLD, corrected for possible confounders, was the primary outcome. The secondary outcomes analyzed were detectable EBV VL, reduction in immunosuppression based on detectable EBV VL, rejection, death, and graft loss (retransplant or death).

Statistical Analysis

For each of the 4 groups, continuous variables (covariates) were reported as means with SDs and binary (yes or no) categorical variables as percentages or proportions. In the assessment of the effect of monitoring on the occurrence of PTLD, to deal with the issue of relatively many potential confounders versus a relatively small number of PTLD events, we replaced the set of potentially confounding variables by propensity scores, which were estimated by logistic regression with all relevant baseline variables included. A propensity score is the probability of a patient to be assigned to a particular treatment given his or her baseline variable values. Because there were 4 groups, multinomial logistic regression, with linear main effects and no interactions, with group as a 4-category outcome variable, and all binary baseline variables as predictors, was used to calculate

Table 1. Baseline Characteristics of Patients With Liver Transplant (*n* = 1281) by EBV VL Monitoring Group

Characteristic	EBV VL Monitoring, Contemporary Center M (Group M1) (*n* = 302)	No EBV VL Monitoring, Contemporary Center C (Group C1) (*n* = 579)	No EBV VL Monitoring, Historical Center M (Group M0) (*n* = 116)	No EBV VL Monitoring, Historical Center C (Group C0) (*n* = 284)
Mean age (SD), y	53.2 (11.2)	49.7 (12.5)	47.3 (10.6)	46.2 (12.4)
Male, *n* (%)	217 (71.9)	369 (63.7)	82 (70.7)	151 (53.2)
Underlying liver disease, *n* (%)				
Cirrhosis, posthepatitis*	131 (43.4)	200 (34.5)	48 (41.4)	122 (43.0)
Cholestatic liver disease†	47 (15.6)	158 (27.3)	31 (26.7)	81 (28.5)
Hepatocellular carcinoma as primary indication	103 (34.1)	129 (22.3)	21 (18.1)	23 (8.1)
Acute liver failure	5 (1.7)	61 (10.5)	5 (4.3)	50 (17.6)
Other	16 (5.3)	31 (5.4)	11 (9.5)	8 (2.8)
EBV IgG positive recipient, *n* (%)	290 (96.0)	560 (96.7)	115 (99.1)	278 (97.9)
Initial immunosuppression, *n* (%)				
Cyclosporine	34 (11.3)	65 (11.2)	104 (89.7)	148 (52.1)
Tacrolimus	267 (88.4)	508 (87.7)	12 (10.3)	98 (34.5)
Azathioprine	2 (0.7)	3 (0.5)	42 (36.2)	68 (23.9)
Mycophenolate mofetil	39 (12.9)	164 (28.3)	30 (25.9)	2 (0.7)
Basiliximab	301 (99.7)	562 (97.1)	66 (56.9)	86 (30.3)

EBV = Epstein–Barr virus; VL = viral load.
* Cirrhosis due to viral hepatitis, autoimmune hepatitis, alcoholic hepatitis, nonalcoholic steatohepatitis, metabolic (α-1 antitrypsin deficiency, Wilson disease, and hemochromatosis), or cryptogenic.
† Cholestatic liver disease with or without cirrhosis due to primary biliary cholangitis, primary sclerosing cholangitis, secondary sclerosing cholangitis, Caroli disease, Byler disease (progressive familial intrahepatic cholestasis), or cystic fibrosis.

these propensity scores, which are, for each patient, the probabilities of being in each of the 4 groups on the basis of the values of covariates or binary categorical variables. Thus, each patient had 4 scores, adding up to 1 (100%). From these propensity scores, for each patient, an inverse probability of treatment weight (IPTW) was derived as the inverse of the probability of being in the group that the patient was actually in. Inverse probability of treatment weights were used to achieve a balance among the 4 groups. It should be emphasized that weighting with IPTWs creates an artificial pseudosample of patients with total numbers that need not be equal to the original sample size.

We used cumulative incidences and associated SEs from an unstandardized Kaplan–Meier failure plot and from an IPTW-weighted Kaplan–Meier failure plot calculation to examine differences over time in M1 versus M0 and in C1 versus C0 separately. We compared the difference in M1 versus M0 versus the difference in C1 versus C0 ("(M0 − M1) − (C0 − C1)") weighting with IPTW. These differences were calculated at the 5-, 10-, and 15-year follow-ups and expressed as point estimates with 95% CIs.

The cumulative incidences of patients with detectable EBV VL, immunosuppression reduction based on detectable EBV VL, rejection, death, and graft loss were analyzed by Kaplan–Meier survival analysis.

SPSS Statistics for Windows, version 24.0 (IBM Corporation), and SAS, version 9.4 (SAS Institute), were used for the analyses.

Because of the retrospective nature of the study with existing data and the consent of patients to use the data, the institutional review board waived the need for further consent. This study complied with the latest version of the Declaration of Helsinki. The data will be made available on request.

Role of the Funding Source

This study was not funded by any grants.

RESULTS

Study Population

Of the 1341 patients, 60 were excluded because of follow-up less than 2 weeks–none in the M1 group, 6 in the M0 group, 23 in the C1 group, and 31 in the C0 group; none of these patients developed PTLD. A total of 1281 consecutive patients who met the inclusion criteria with the first LT and a follow-up of more than 2 weeks were included in this study. Table 1 shows the baseline characteristics of LT patients considered important in the analysis of PTLD occurrence.

Examination of Baseline Characteristics

Despite some differences in baseline characteristics, with more females in C0 and some differences in the cause of liver disease, the M1 and C1 groups were largely similar. In the M0 and C0 groups, predominantly cyclosporine and no tacrolimus were used, and basiliximab was used only in 43.7% of patients, whereas in both the C1 and M1 groups, all patients received basiliximab induction and most received tacrolimus. There was no difference in EBV seroprevalence (96.0% to 99.1%). Because of the earlier start of the transplant program, the median follow-up in the C0 group was longer than in the M0 group (16.0 vs. 11.9 years). The follow-up period did not differ between the M1 and C1 groups.

Monitoring of EBV VL and Immunosuppression Reduction

In the 302 patients in the M1 group, a median of 14 EBV VL measurements per patient (interquartile range, 10 to 18; range, 0 to 39; total, 4461) were done in the first year after LT, and after the first year after LT a median of 11 EBV VL measurements per patient were done (interquartile range, 6 to 16; range, 0 to 71; total, 3891). The cumulative incidence of reported first measurable EBV VL

during post-LT survival in the M1 group and the cumulative incidence and timing of immunosuppression reduction for a measurable EBV VL are shown in Supplement Figure 1. From the 35 of 302 (12%) patients with 2 or more positive EBV VL measurements within 2 months within the first year, 31 (89%) had immunosuppression reduction as required per protocol and 4 (11%) did not. Out of 64 of the 302 patients (21.2%) with 1 measurable EBV VL followed by an undetectable EBV VL within the first year, 28 (44%) had a reduction in immunosuppression based on the judgment of the physician and 36 (56%) did not. Therefore, during the first year from 99 of 302 (33%) patients with 1 or more detectable EBV VLs, 59 (60%) received a reduction of immunosuppression and 40 (40%) did not. In 5 of these cases, positive EBV VL was detected during antirejection treatment and in 3 cases during induction immunosuppression immediately after the first LT (2 cases) or retransplant (1 case).

After the first year, 43 of 302 (14%) patients had a single positive EBV VL, which was followed by a reduction in immunosuppression in 16 of 43 (37%) patients and no reduction in immunosuppression in 27 of 43 (63%) patients. After the first year, 31 of 302 (10%) had multiple positive EBV VLs, which was followed by a reduction in immunosuppression in 18 of 31 (58%) cases and no reduction in immunosuppression in 13 of 31 (42%) cases. Therefore, in total, after the first year, 1 or more positive EBV VL cases occurred in 74 of the 302 (25%) patients, which led to a reduction in immunosuppression in 34 of 74 (46%) of these patients, whereas 40 of these 74 (54%) patients had no reduction in immunosuppression after detectable EBV VL after the first year. Of these 34 patients with reduction of immunosuppression based on detectable EBV VL after the first year, 7 had reduced immunosuppression in the first year after LT for 2 or more detectable EBV VLs, 3 had reduced immunosuppression in the first year for 1 detectable EBV VL, and 24 had no reduction of immunosuppression for detectable EBV VL during the first year after LT. In two cases, EBV VL only became undetectable with rituximab.

Further details on the reduction of immunosuppression for detectable VLs are shown in the Supplement.

Monitoring of EBV VL and Incidence of PTLD

The crude incidence rates (number of events/total follow-up time per group) were as follows for the 4 groups: EBV VL monitoring strategy group from center M (M1): 1 per 2228.9 person-years; contemporary control group from center C without EBV VL monitoring strategy (C1): 10 per 4143.5 person-years; historical group from center M without EBV VL monitoring strategy (M0): 8 per 1289.9 person-years; and historical group from center C without EBV VL monitoring strategy (C0): 10 per 3611.8 person-years. Of the 29 PTLD cases, 14 were EBV related, 8 were EBV unrelated, and in 7 this was unknown; 1 case in the M1 group was a stage 1 polyclonal polymorphic PTLD, and all PTLDs in the other groups were M-PTLDs (Supplement Table).

Table 1 shows the baseline characteristics considered important for the LT patients in the analysis of PTLD occurrence. However, the distributions of the IPTW scores showed many influential outliers (Supplement Figure 2). Therefore, we trimmed the IPTW scores such that scores above the 95th percentile in each group were replaced by the 95th percentile, and scores below the 5th percentile were made equal to the 5th percentile (Supplement Figure 3). These trimmed IPTWs were used to calculate IPTW-weighted means and percentages. In Table 2, the result of the weighting with the trimmed IPTWs overall shows improvement among the 4 groups compared with the percentages in Table 1, but a complete balance has not been achieved.

The Figure shows the IPTW-weighted cumulative incidences as failure Kaplan–Meier plots for the 4 groups. Table 3 shows the cumulative incidences expressed as the number of occurrences per 1000 patients at 5-, 10-, and 15-year follow-up for the 4 groups in the unstandardized and standardized analysis, respectively. The historical era shows almost consistently more PTLD events

Table 2. Standardized Means or Proportions of Baseline Patient Characteristics* Based on Inverse Probability of Treatment Weights

Baseline Variable	EBV VL Monitoring Contemporary Center M (Group M1)	No EBV VL Monitoring Contemporary Center C (Group C1)	No EBV VL Monitoring Historical Center M (Group M0)	No EBV VL Monitoring Historical Center C (Group C0)
Mean age, y	51.00	50.30	47.10	48.80
Male	0.67	0.66	0.63	0.63
Underlying liver disease				
Cirrhosis, posthepatitis	0.40	0.39	0.28	0.44
Cholestatic liver disease	0.24	0.24	0.46	0.26
Hepatocellular carcinoma as primary indication	0.26	0.25	0.11	0.17
Acute liver failure	0.04	0.08	0.07	0.10
Other	0.06	0.05	0.07	0.03
EBV IgG positive recipient	0.97	0.97	0.97	0.96
Initial immunosuppression				
Cyclosporine	0.15	0.15	0.39	0.31
Tacrolimus	0.83	0.81	0.61	0.69
Azathioprine	0.01	0.01	0.12	0.13
Mycophenolate mofetil	0.22	0.22	0.31	0.03
Basiliximab	0.99	0.95	0.75	0.74

EBV = Epstein–Barr virus; VL = viral load.
* See Table 1.

Figure. Inverse probability of treatment weighted cumulative incidence of PTLD by group.
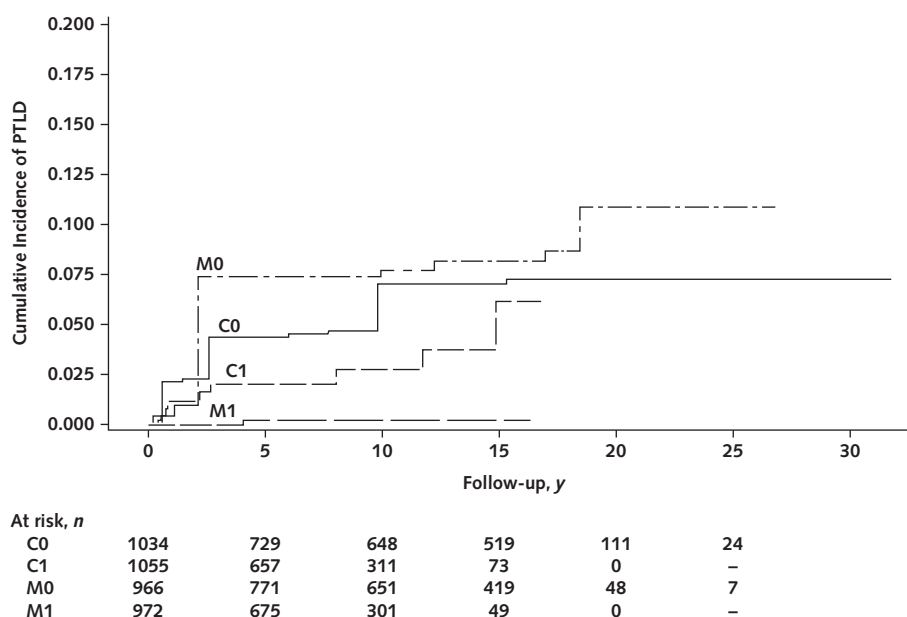
The graph shows inverse probability of treatment weighted number of patients at risk at 5-y intervals (Kaplan–Meier 1-survival curves) for contemporary EBV monitoring group from center M (M1), historical group from center M without EBV monitoring (M0), contemporary control group from center C without EBV monitoring (C1), and historical group from center C without EBV monitoring (C0). EBV = Epstein–Barr virus; PTLD = posttransplant lymphoproliferative disease.

than the recent era in both centers (positive values for M0 − M1 and C0 − C1), but the 95% CIs include the null value of 0. The difference in the differences (last column in the table) shows positive numbers for all follow-up time points. For the IPTW-weighted analysis, monitoring shows the most favorable outcome at 15-year follow-up, with an estimate of 70.6 fewer PTLD cases per 1000 patients. However, the 95% CI (−61.7 to 202.9) includes the 0. Thus, the CI ranges from an increase in the monitoring group of 61.7 cases per 1000 patients to a decrease of 202.9 cases per 1000 patients.

Rejection Treatment in Contemporary Groups

As shown in the **Supplement**, the proportion of patients with rejection treatment, occurring especially after the first 3 months in the first year after LT, seemed to be higher in the M1 group than in the C1 group. All of these rejections responded to glucocorticoids and increased baseline immunosuppression only. Rejection seemed to be more frequent in historical group C0 than in contemporary group C1 and in historical group M0 than in contemporary group M1. Additional data are shown in the **Supplement**.

DISCUSSION

After LT in adults, the difference-in-difference analysis showed a numerically larger within-hospital decrease in PTLD in the M hospital—with EBV VL monitoring strategy in the contemporary cohort—over time than in the C hospital—without EBV VL monitoring strategy.

A decreasing incidence of PTLD over time has been mentioned in previous literature and is likely to be related to less immunosuppression in contemporary versus historical patients, similar to renal transplant (2, 31). The current data suggest that EBV VL monitoring may be associated with less PTLD but more rejection in the first year, which could be associated with a reduction in immunosuppression; all of these rejections were easily treatable and did not lead to graft loss.

Posttransplant lymphoproliferative disease is associated with morbidity and mortality (1, 2, 32, 33). Therefore, a strategy that could reduce the incidence of PTLD would be of utmost importance. Approximately 70% of PTLD is EBV related. In adult LT cohorts, more than 90% of recipients are EBV seropositive compared with about 50% in children, leading to less early EBV primary infection and more long-term EBV reactivation, similar to stem cell transplant (34). This partially explains why, in adults, PTLD presentation is often delayed after transplant (1–3,10). Indeed, in the contemporary control group, after a slightly higher incidence in the first year, there was a low but constant incidence of PTLD over several years.

In the current study, in about half of the patients, EBV VL was detected during long-term follow-up, whereas in a recent study on LT, this occurred in 70% of patients (35). The current data contradict the conclusion from that study that EBV viremia is benign (35), and on the basis of the current data, we consider detectable EBV VL as a sign of overimmunosuppression, which can lead to B-lymphocyte proliferation and PTLD. With an EBV VL monitoring strategy, the lowered immunosuppression, which in most cases

Table 3. Unstandardized and Standardized (Inverse Probability of Treatment Weighted) Cumulative Incidences of Posttransplant Lymphoproliferative Disease per 1000 Patients*

Cumulative Incidences	C0	C1	M0	M1	C0 − C1	M0 − M1	(M0 − M1) − (C0 − C1)
Unstandardized							
5-y cumulative incidence (95% CI)	25.5	13.3	40.4	4.2	12.2 (−10.2 to 34.6)	36.2 (−3.5 to 75.8)	24.0 (−21.6 to 69.5)
10-y cumulative incidence (95% CI)	41.5	17.7	40.4	4.2	23.8 (−6.0 to 53.6)	36.2 (−3.52 to 75.8)	12.4 (−37.3 to 62.0)
15-y cumulative incidence (95% CI)	41.5	51.3	71.1	4.2	−9.8 (−68.8 to 49.2)	66.9 (9.81 to 123.9)	76.7 (−5.4 to 158.7)
Standardized							
5-y cumulative incidence (95% CI)	43.5	20.0	74.2	2.7	23.5 (−17.8 to 64.8)	71.5 (−30.3 to 173.3)	48.0 (−61.8 to 157.9)
10-y cumulative incidence (95% CI)	69.9	27.5	71.8	2.7	42.8 (−11.0 to 96.6)	71.5 (−30.3 to 173.3)	28.7 (−86.4 to 143.8)
15-y cumulative incidence (95% CI)	70.4	61.9	81.8	2.7	8.5 (−69.3 to 84.1)	79.1 (−30.5 to 182.9)	70.6 (−61.7 to 202.9)

* The differences within hospital and 95% CIs, and the difference of the 2 within-hospital differences (95% CI), at 5-, 10-, and 15-y follow-up. C0 = Historical Group From Center C Without EBV VL Monitoring; C1 = Contemporary Control Group From Center C Without EBV VL Monitoring; EBV = Epstein–Barr virus; M0 = Historical Group From Center M Without EBV VL Monitoring; M1 = Contemporary EBV VL Monitoring Group From Center M; VL = viral load.

was maintained long term, may have rendered EBV VL undetectable without negative effects and may have reduced the incidence of PTLD. A spontaneous return to undetectable EBV VL also occurred, which may be because of a booster of anti-EBV immunity by EBV reactivation and which may protect against PTLD.

There are limitations to the findings and conclusions of this study. The level of EBV VL above which action is required has not been well established. In the current monitoring cohort, we chose to perform persistently detectable EBV VL. Assays and samples other than the EDTA plasma used in this study or even use of the same assay in a different laboratory may yield another cutoff for the detection limit (36). The balance among the groups after IPTW weighting was not ideal. In addition, the number of outcome events was limited. Thus, the comparison was limited by low statistical power in the current study: All relevant 95% CIs for differences and for differences in differences in cumulative incidences included the null value. This is not unexpected, partly because restriction of the between-group comparisons to a specific point in time–for example, 5- or 10-year follow-up–may lead to some loss of statistical "power" to show (absolute) differences. In addition, we emphasize that the only PTLD case in the monitoring group was polyclonal polymorphic PTLD, whereas the PTLD cases in the other groups were all of the M-PTLD type. An analysis of only M-PTLD cases, resulting in no PTLD cases in the monitoring group, would have shown an even more favorable result for the monitoring group. Ideally, this would have been a cluster or center randomized trial yielding many more cases of PTLD instead of an observational study in 2 centers. Another limitation is that although the EBV VL monitoring strategy in the first year was according to the protocol in 89% of the cases, this was not standardized after the first year. The optimal number of EBV VL measurements per year is unknown, but measuring more frequently is probably better. The analysis was retrospective; therefore, although unlikely, early lesions may have been missed, and EBV-negative PTLD may not have been prevented by this strategy. The strengths of this study include the contemporary control group, standardization by IPTW analysis, and long-term

follow-up. Because of the limitations, there is a need for future larger studies to further evaluate the association between an EBV VL monitoring strategy and possible prevention of PTLD more definitively.

Despite these limitations, we strongly believe that the reported results merit serious consideration of the EBV VL monitoring policy in an attempt to reduce the incidence of PTLD after LT in adults. At least such a strategy seems safe. An EBV VL monitoring strategy with immunosuppression reduction may reduce the incidence of PTLD in other adult patients with long-term immunosuppression and may contribute to tumor surveillance and prevention of other infections; however, future studies should confirm this.

From Department of Gastroenterology and Hepatology, LUMC Transplant Center, Leiden University Medical Center, Leiden, the Netherlands (B.N.R., M.H., M.E.T., B.v.H.); Department of Biomedical Data Sciences, Medical Statistics, Leiden University Medical Center, Leiden, the Netherlands (R.W.); Department of Surgery, Erasmus MC Transplant Institute, Erasmus Medical Center, Rotterdam, the Netherlands (M.v.R.); Department of Gastroenterology and Hepatology and Department of Surgery, LUMC Transplant Center, Leiden University Medical Center, Leiden, the Netherlands (D.v.d.H.); Department of Surgery, LUMC Transplant Center, Leiden University Medical Center, Leiden, the Netherlands (J.D.); Department of Gastroenterology and Hepatology, Erasmus MC Transplant Institute, Erasmus Medical Center, Rotterdam, the Netherlands (H.M.); and Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands (A.C.T.M.V.).

Corresponding Author: Bart van Hoek, MD, PhD, Department of Gastroenterology and Hepatology, C4-P, and Transplantation

Center, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, the Netherlands; e-mail, b.van_hoek@lumc.nl.

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