ORIGINAL ARTICLE

Risk factors for Epstein–Barr virus reactivation after renal transplantation: Results of a large, multi-centre study

Arturo Blazquez-Navarro[1,2,3] (iD), Chantip Dang-Heine[4], Patrizia Wehler[2,3], Toralf Roch[2,3], Chris Bauer[5], Sindy Neumann[6], Rodrigo Blazquez-Navarro[1], Andriy Kurchenko[7], Kerstin Wolk[2,14], Robert Sabat[2,14], Timm H. Westhoff[3], Sven Olek[8], Oliver Thomusch[9], Harald Seitz[10], Petra Reinke[2,11], Christian Hugo[12] (iD), Birgit Sawitzki[2], Michal Or-Guil[1,13] (iD) & Nina Babel[2,3] (iD)

1 Systems Immunology Lab, Department of Biology, Humboldt-Universität zu Berlin, Berlin, Germany
2 Berlin Institute of Health Center for Regenerative Therapies (BCRT): Berlin-Brandenburger Centrum für Regenerative Therapien, Charité – Universitätsmedizin Berlin, Berlin, Germany
3 Center for Translational Medicine, Universitätsklinikum der Ruhr-Universität Bochum, Medizinische Klinik I, Herne, Germany
4 Clinical Study Center (CSC), Berlin Institute of Health, and Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin, Germany
5 MicroDiscovery GmbH, Berlin, Germany
6 numares AG, Regensburg, Germany
7 Department of Clinical Immunology and Allergology, Bogolomets National Medical University, Kiev, Ukraine
8 Ivana Türbachova Laboratory for Epigenetics, Epiontis GmbH, Precision for Medicine Group, Berlin, Germany
9 Klinik für Allgemein- und Viszeralchirurgie, Universitätsklinikum Freiburg, Freiburg, Germany
10 Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics und Bioprocesses, Leipzig, Germany
11 Berlin Center for Advanced Therapies (BeCAT), Charité –

SUMMARY

Epstein–Barr virus (EBV) reactivation is a very common and potentially lethal complication of renal transplantation. However, its risk factors and effects on transplant outcome are not well known. Here, we have analysed a large, multi-centre cohort ($N = 512$) in which 18.4% of the patients experienced EBV reactivation during the first post-transplant year. The patients were characterized pre-transplant and two weeks post-transplant by a multi-level biomarker panel. EBV reactivation was episodic for most patients, only 12 patients showed prolonged viraemia for over four months. Pre-transplant EBV shedding and male sex were associated with significantly increased incidence of post-transplant EBV reactivation. Importantly, we also identified a significant association of post-transplant EBV with acute rejection and with decreased haemoglobin levels. No further severe complications associated with EBV, either episodic or chronic, could be detected. Our data suggest that despite relatively frequent EBV reactivation, it had no association with serious complications during the first post-transplantation year. EBV shedding prior to transplantation could be employed as biomarkers for personalized immunosuppressive therapy. In summary, our results support the employed immunosuppressive regimes as relatively safe with regard to EBV. However, long-term studies are paramount to support these conclusions.

Universitätsmedizin Berlin, Berlin, Germany

12 Universitätsklinikum Carl Gustav Carus, Medizinische Klinik III - Bereich Nephrologie, Dresden, Germany

13 Institute of Medical Immunology, Charité – Universitätsmedizin Berlin, Berlin, Germany

14 Psoriasis Research and Treatment Center, Charité – Universitätsmedizin Berlin, Berlin, Germany

Correspondence

Nina Babel. Center for Translational Medicine, Universitätsklinikum der Ruhr-Universität Bochum, Medizinische Klinik I Hölkeskampring 40, 44625 Herne, Germany.
Tel.: +49 2323 4995141; Fax : +49 2323 4993302;
e-mail:
nina.babel@elisabethgruppe.de

Introduction

Infections with viral pathogens can result in major complications after solid organ transplantation. Immunosuppression can disrupt the control of latent infections in organ recipients and increase the risk of transmission from the donor or the general population, leading to life-threatening viral infections and reactivations [1,2]. One of the most frequently occurring viral pathogen in renal transplantation is the Epstein–Barr virus (EBV) [3,4]. Approximately 90% of the population are latently infected with EBV in high- and middle-income countries [5–7]. After transplantation, EBV primary infections and reactivations posit a serious risk known as post-transplant lymphoproliferative disorders (PTLD), ranging from asymptomatic self-contained viraemia, to potential lethal, malignant disease [8,9].

In our previously published work derived from the Harmony cohort, we found a 20% prevalence of EBV viraemia either pre-transplant or during the first post-transplant year [3,4]. The main risk factors for EBV viraemia were found to be rabbit antithymocyte globulin (rATG) induction treatment, as well as EBV mismatch (donor seropositive, recipient seronegative – D+/R−) and cytomegalovirus D+/R− mismatch [3,4]. However, several aspects of the EBV viraemia course are still unclear. For example, the relationship between pre-transplant and post-transplant viraemia has not been sufficiently studied. Importantly, no reliable models for

the early prediction of EBV reactivation to assist therapeutic decisions currently exist [10].

In this study, we perform a further and more detailed characterization of EBV reactivation within our well-characterized Harmony cohort [3,4]. Here, we provide evidence on the significance of pre-transplant EBV viral load for the transplant course as well as risk factors for EBV reactivation potentially enabling personalization of immunosuppressive therapy. Furthermore, we offer evidence that the employed immunosuppressive regimes were relatively safe regarding severe EBV-associated complications.

Methods

Patient population and medication

We have conducted a sub-study within the randomized, multi-centre, investigator-initiated Harmony trial (NCT 00724022) to determine the risk factors and impact of EBV reactivation [4,11]. Thus, the patients were characterized for EBV reactivation and clinical markers pre-transplant and at seven post-transplant visits [4]. The study was carried out in compliance with the Declaration of Helsinki and Good Clinical Practice.

As described previously, the patient cohort received one of three immunosuppressive therapy regimes [11]. Thus, patients received an induction therapy with basiliximab or rATG. Maintenance therapy consisted of

tacrolimus, mycophenolate mofetil (MMF) and corticosteroids, whereas for some patients, corticosteroids were withdrawn at day 8. According to the study protocol, patients with D+/R- mismatch for either CMV or EBV as well as all patients with rATG induction, received a valganciclovir (VGCV) prophylaxis [11]. VGCV prophylaxis was defined as VGCV treatment initiated during the first 14 days, as explained before [12]. Reported MMF dose and tacrolimus trough levels correspond to this same 14 day threshold.

Monitoring of viral load

Patients were monitored for transplant outcomes during the first post-transplant year. Thus, peripheral blood samples were centrally monitored for EBV, cytomegalovirus (CMV) and BK virus (BKV) by TaqMan qPCR along eight visits, as described previously [4]. Shortly, blood samples were taken at day 0 (pre-transplant, pre-Tx), 2nd week (2w), 1st month, 2nd month, 3rd month, 6th month, 9th month and 12th month post-transplant. DNA was isolated from whole blood samples, for EBV and CMV, or serum, for BKV, following the manufacturer's instructions. The qPCR was performed employing the Prism 7700 Sequence Detector. The detection level was determined as the lowest viral load measured within the range of linearity (250 copies/ml).[4]

Duration of EBV viraemia was calculated as the time between the first and the last post-transplant samples with detectable EBV load, including possible episodes of negative viral load in-between. Patients with an EBV duration over 120 days were classified as EBV_{chron}. Based on their peak viral load values for EBV, the patients were classified as follows: patients with at least one viral load measurement over detection limit were classified as EBV^+; patients with at least one viral load measurement over 1000 copies/ml were classified as $EBV_{increased}$; patients with no viral load measurement over detection limit were classified as EBV^-; lastly, patients with EBV viral load over detection limit at pre-Tx were classified as pre-Tx-EBV^+.

Monitoring of transplant outcomes

The patient cohort was monitored at the local centres at the eight pre-defined visits. Thus, estimated glomerular filtration rate (eGFR), as well as full blood count and routine chemistry tests were estimated. eGFR was assessed using the CKD-EPI formula, measured in ml/min/1.73 m^2 [13]. Suspected episodes of acute rejection were confirmed through biopsy; histologic characteristics were described according to the Banff criteria of 2005 [4,11,14].

Characterization of marker subsets for EBV viraemia

The patients were characterized for a multi-level biomarker panel consisting of five subsets, at pre-Tx and 2w. Shortly, gene expression markers were measured employing TaqMan Gene Expression Assays (Thermo Fisher Scientific). Leucocyte subsets were determined based on the epigenetic quantification, as described before [15,16]. Screening of serum antibody binding profile (SAB) was performed employing HLA-1 mixed antigen bead assay; the raw mean fluorescence intensity for each bead was employed for prediction [17]. The urinary metabolomics spectrum was measured by nuclear magnetic resonance (NMR) spectroscopy at numares AG (Regensburg, Germany). Finally, clinical data were measured de-centrally and were provided by the transplantation centres. For more details on the methods and the list of measured markers, see the Supplementary Methods.

Statistical analysis

Prediction of EBV reactivation and identification of risk factors were performed for each of the five pre-defined marker subsets, employing data from pre-Tx and 2w. Here, EBV^+ patients were compared with EBV^- and $EBV_{increased}$ with EBV^-. Patients with detectable EBV load during or before sampling were disregarded for the analysis, as we aimed for prognostic models and risk factors.

Prediction of EBV viraemia was performed employing the machine learning algorithm random forest for classification (R package randomForest, version 4.6-14) with cross-validation [18]. The performance of the EBV classification was assessed by the balanced accuracy (BACC).

For the analysis of individual EBV risk factors, we did not include the SAB and the urine metabolome subset, since these are to be interpreted as a whole rather than as individual markers. Markers demonstrating a significant difference between the viraemia sub-cohort and the EBV^- were considered as potential EBV prognostic markers. The significance of this difference was tested using the two-tailed Mann–Whitney test for quantitative factors and two-tailed Fisher's exact test for categorical factors. Adjusted *P* values were calculated employing the Benjamin & Hochberg method.

Categorical variables are summarized here as numbers and frequencies; quantitative variables are reported as median and interquartile range (IQR). The significance of an association with EBV reactivation was tested

employing Mann–Whitney or Fisher test, as for risk factors. Correlations were evaluated employing Spearman's rank correlation. Forest plots depict the odds ratio (OR) as square point and 95% confidence interval (95% CI) as a line. For more details on the statistical methods, see the Supplementary Methods.

Results

Identification of EBV risk factors

Characteristics of EBV reactivation in the cohort

A total of 512 patients were characterized for EBV viraemia: 94 patients (18.4%; hereafter EBV$^+$ group) had post-transplant EBV viral load, 44 patients (46.8%) of them had clinically relevant viral loads of over 1000 copies/ml in blood (from now on EBV$_{increased}$). Patients who did not suffer from detectable EBV reactivation during the first post-transplant year are here referred to as EBV$^-$.

For most patients, viraemia was only episodic. 12 patients suffered from prolonged reactivations of over 4 months (from now on EBV$_{chron}$). These EBV$_{chron}$ patients suffered from particularly severe EBV reactivations, with significantly higher peak viral loads compared with the rest of the EBV$^+$ patients (3198 [1194–6862] vs. 791 [428–2283] copies/ml; $P = 0.010$). In fact, there was a highly significant correlation between reactivation duration and peak viral load ($\rho = 0.37$; $P < 0.001$).

Pre-transplant EBV load is a risk factor for post-transplant EBV reactivation

A total of 373 (72.9%) patients of the cohort were characterized for their pre-transplant EBV load. A substantial number of patients (6.1%, $N = 23$; hereafter pre-Tx-EBV$^+$) showed detectable viral load in blood before

transplant, with a median load of 1064 [810–2764] copies/ml. As expected, pre-Tx-EBV$^+$ patients had a significantly higher incidence of EBV reactivation post-transplant (OR: 4.89 [1.85–12.79], $P = 0.001$). While these patients also had a tendency towards higher viral loads, the difference was not significant (1765 [602–4097] vs. 782 [384–2440] copies/ml; $P = 0.222$). For the duration of reactivation, we found a borderline significant tendency towards longer reactivation among pre-Tx-EBV$^+$ patients (14 [0–110] vs. 0 [0–12] days; $P = 0.082$). Pre-Tx-EBV$^+$ was not significantly associated with EBV$_{chron}$ (OR: 2.53 [0.35–14.42]; $P = 0.351$).

No effective multi-parameter predictive model of EBV reactivation could be identified

Here, we analysed a large number of markers (see Supplementary Methods) at pre-Tx and 2w, with the goal of predicting which patients become are EBV$^+$ or EBV$_{increased}$. First, we analysed the marker subsets as a whole, with the goal of determining whether certain marker types are more adequate for prediction of EBV viraemia. However, none of the marker types was able to predict EBV reactivation satisfactorily (Fig. S1): The best performing marker subset was the SAB profiles at pre-Tx (BACC = 61.1% sensitivity = 33.3%, specificity = 88.9%).

Immunotolerance marker genes, male sex and metabolic alterations were prognostic for EBV reactivation

As no effective multi-parameter predictive model could be generated, we evaluated whether any potential markers for EBV reactivation could be identified. Previously, an association of EBV$^+$ with rATG induction and of EBV$_{increased}$ with D+/R− mismatch for CMV and EBV had been observed [4]. In addition to the previous results, we observed an association of EBV$^+$ with male sex (OR: 1.71 [1.02–2.94]; $P = 0.033$; Figs 1 and S2).
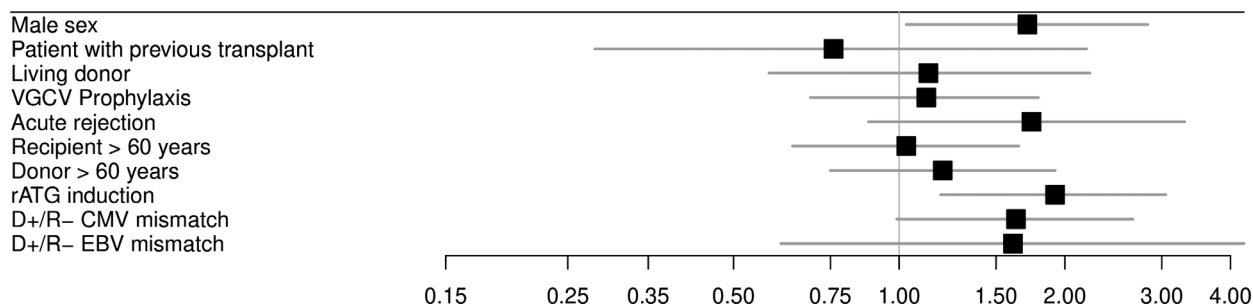

Figure 1 Forest plot comparing EBV$^+$ versus EBV$^-$ patients for selected demographic factors. The square denotes the odds ratio, whereas an odds ratio over 1 means an increased higher incidence among EBV$^+$ patients; the grey lines denote the 95% confidence interval.

Figure 2 Forest plot comparing EBV$^+$ versus EBV$^-$ patients for binary outcomes and transplant complications. The square denotes the odds ratio, whereas an odds ratio over 1 means an increased higher incidence among EBV$^+$ patients; the grey lines denote the 95% confidence interval.
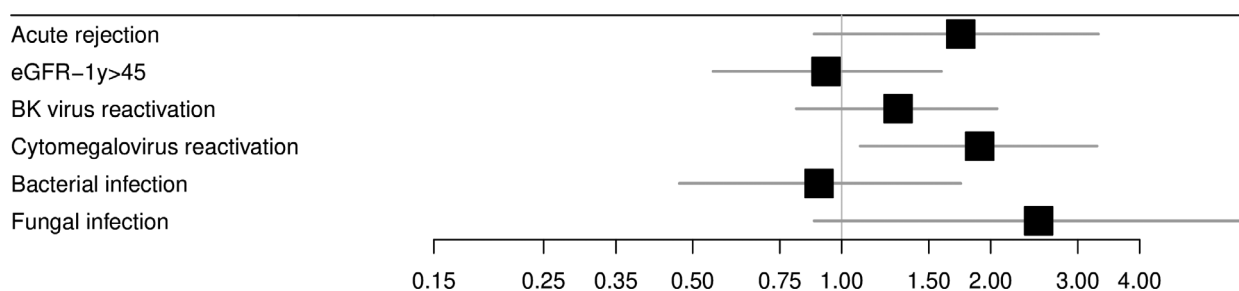
Furthermore, we evaluated our multi-level marker panel to identify potential markers predictive of EBV reactivation (Tables S1 and S2). Only the expression of the genes CD200 and LAG3 – immunological co-inhibitory – at 2w was potentially associated with EBV reactivations (Fig. S3). However, the encountered differences for these genes were not significant when adjusting for multiple testing; none of the measured markers showed a significant association with EBV reactivation after adjustment (see Tables S1 and S2).

Evaluation of the impact of EBV reactivation on post-transplantation

Post-transplant EBV reactivation was associated with higher incidence of acute rejection

We examined the transplant outcome of EBV$^+$ and EBV$_{increased}$ patients one year after transplant (Figs 2 and S2), as well as the incidence of opportunistic infections. Interestingly, we observed an increased incidence of acute rejection among EBV$_{increased}$ (OR: 2.70 [1.11–6.10]; $P = 0.019$). Furthermore, we analysed whether a particular order of events in the association of acute rejection with EBV can be determined. Of 15 EBV$^+$ patients with acute rejection, 10 (66.7%) patients demonstrated viraemia after the first acute rejection episode. Three patients (20.0%) had both adverse events within a time span of 2 weeks.

No significant effect of EBV was observed on eGFR one year post-transplant, nor on the incidence of loss to follow-up. EBV$^+$ patients had a significantly higher incidence of CMV reactivation, while no clear effect association between EBV and BKV reactivation could be detected [4]. We did not observe a significant increase of fungal or bacterial opportunistic infections among EBV$^+$ patients nor EBV$_{increased}$ (Figs 2 and S2). Regarding the occurrence of anaemia, we observed within the EBV$^+$

sub-cohort a significant negative correlation ($\rho = -0.29$; $P = 0.009$) between EBV viral load and haemoglobin levels one year after transplantation (Fig. 3).

Patients with prolonged EBV reactivation had a higher incidence of BKV reactivation

We investigated whether the 12 patients with EBV$_{chron}$ had distinctive transplant outcomes or complications, compared with the rest of the EBV$^+$ sub-group. There was no evidence of alterations of the graft function; no difference was observed in the incidence of acute rejection, CMV, bacterial and fungal infections either (data not shown). However, there was an increase in BKV reactivation incidence (OR: 4.69 [0.92–46.67]; $P = 0.059$), when comparing EBV$_{chron}$ with the rest of the EBV$^+$ sub-group.

Discussion

We have performed a characterization of the risk factors and associated complications of EBV reactivation in the context of renal transplantation. Despite relatively frequent reactivation of EBV during the first post-transplant year, no clear worsening of transplantation outcome was observed. Only a significant association with increased incidence of acute rejection and a correlation with low haemoglobin values were observed. Furthermore, we identified pre-transplant EBV shedding, male sex and, tentatively, increased LAG3 and CD200 gene expression two weeks post-transplant as potential predictors of EBV reactivation.

One of our main findings is the importance of pre-transplant EBV load as a post-transplant predictor. Self-limiting, asymptomatic EBV shedding in saliva or blood is common phenomena in healthy individuals, with great degree of inter- and intra-individual variation [19–21]. Importantly, both renal insufficiency and haemodialysis have a systemic impact on the immune
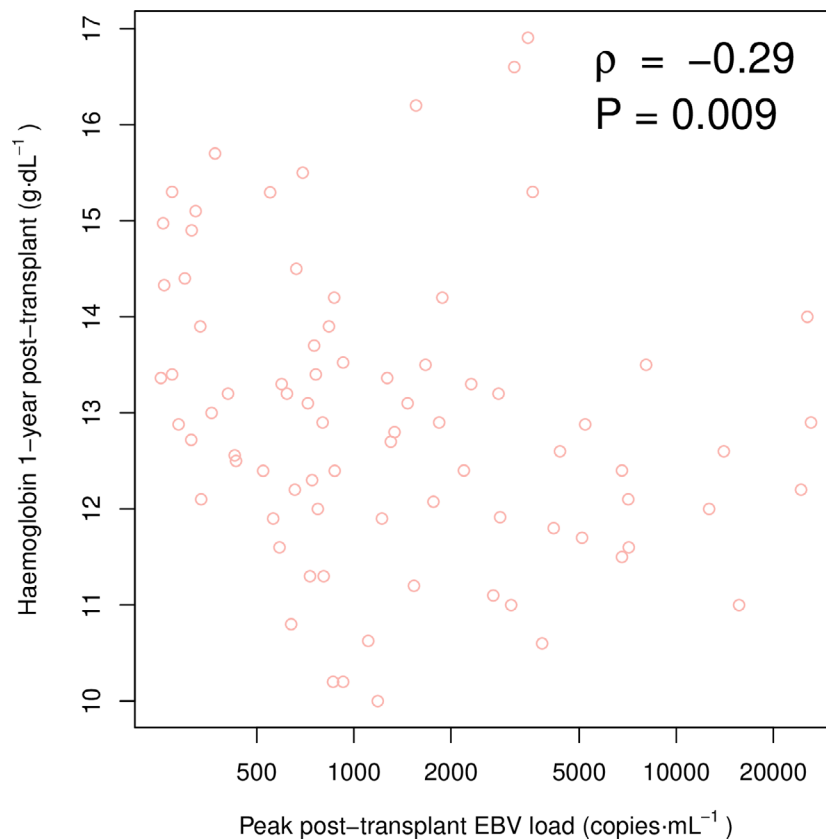
Figure 3 Correlation of peak viral among EBV$^+$ patients with haemoglobin values one year post-transplant. Haemoglobin values below 14 g/dl for males and below 12 g/dl for females are considered indicative for anaemia.

response, potentially leading to reactivation events of latent pathogens [22,23]. Because of this, a substantial incidence of EBV pre-transplant shedding was expected [10]. Verghese *et al.*, to our knowledge the only study in this topic in the literature, did not observe any such association between pre-transplant shedding and post-transplant reactivation. On the other hand, in our study the patients with pre-transplant EBV shedding were prone to four times higher incidence of post-transplant reactivation [10]. The reason for the discrepancy could lie on the small sample analysed by Verghese *et al* [10].

Of note, our observed values for pre-transplant EBV incidence are remarkably similar to those by Verghese *et al.* Thus, they found a prevalence of 5%, compared with 6% in our study. The observed post-transplant incidence was also very similar – 21% vs. 18%. Regarding other studies on post-transplantation incidence, a wide range of EBV incidences (13–48%) has been observed after solid organ transplantation [3,19,24–26]. The large degree of inter-study variation can be because of differences in immunosuppressive regimes as well as the use of non-standardized quantitative PCR tests in several studies in the literature (including this work). The latter

complicates the comparison between the results of the literature. However, it should be noted that our observed incidence for post-transplant EBV was in the lower range of the literature, so that an overestimation of EBV incidence seems unlikely [3,19,24–26].

We also identified potential associations of EBV reactivation with clinical alterations. We detected a significant correlation of EBV viral load with reduced haemoglobin levels. Furthermore, a significantly increased incidence of acute rejection was found. This is in line with our previous report demonstrating an association between EBV reactivation and transplant rejection [27]. With respect to their timeline, our data suggest that acute rejection precedes EBV reactivation in most cases. Anti-rejection therapy might play an important role in diminishing immune surveillance and accelerating disruption of EBV latency in patients with acute rejection.

Our data showed a tendency towards increased expression of the genes LAG3 and CD200 before EBV viraemia. These genes play a role in the regulation of the immune response and have a co-inhibitory effect on T-cell-mediated immunity [28,29]. A potential association with opportunistic infections such as EBV would

Table 1. Characteristics of the patient cohort. These include information on demographics, treatment and transplant outcome. Categorical variables are shown as count (% frequency), continuous variables as median [IQR].

Variable	Variable type	Cohort (N = 512)
Male sex	Categorical	327 (63.9%)
Age at pre-Tx (years)	Continuous	56 [46–64]
Weight at pre-Tx (kg)	Continuous	77.2 [68.1–88.6]
BMI at pre-Tx (kg/m^2)	Continuous	25. 8 [23.2–29.0]
Patient with previous transplant	Categorical	490 (95.7%)
Living donor	Categorical	65 (12.7%)
Age of donor (years)	Continuous	55 [46–65]
Expanded criteria donor*	Categorical	221 (43.2%)
Total HLA mismatches	Continuous	3 [2–4]
Cold ischaemia time (min)	Continuous	619 [416–840]
Immunosuppression		
basiliximab + MMF + tacrolimus + corticosteroids	Categorical	185 (36.1%)
basiliximab + MMF + tacrolimus	Categorical	166 (32.4%)
rATG + MMF + tacrolimus	Categorical	161 (31.4%)
VGCV Prophylaxis	Categorical	299 (58.4%)
Acute rejection	Categorical	56 (10.9%)
Severe BKV viraemia (>10,000 copies/ml)	Categorical	59 (11.5%)
Severe CMV viraemia (>10,000 copies/ml)	Categorical	18 (3.5%)
eGFR-2w (ml/min·1.73 m^2)	Continuous	32.1 [18.1–46.2]
eGFR one year post-transplant (ml/min·1.73 m^2)	Continuous	47.6 [35.0–60.8]

*The expanded criteria comprise a donor age greater than 60 years, or an age greater than 50 years combined with at least two of the following factors: cerebrovascular accident as the cause of death, hypertension, or a serum creatinine level of more than 1.5 mg/dl [11].

therefore be not surprising. In fact, CD200 is differentially expressed in EBV-associated PTLD, while LAG3 is highly expressed in EBV$^+$ gastric cancers [30,31]. However, it should be noted that no significant association of the genes with EBV viraemia was observed when adjusting for multiple testing. Therefore, further studies are paramount to confirm this hypothetical association. Regarding sex effects, male patients suffered from a significantly higher incidence of EBV reactivation in our cohort. This is in disagreement with previous epidemiological studies with smaller cohorts [32–34].

There are limitations in our study. First, no correction for multiple testing was performed outside of the marker panel analysis, with the goal of capturing the maximum number of potential associations [35–37]. Therefore, the results we present here should be reproduced in confirmatory studies to consider them definitive. Secondly, this study was performed on a low immunological risk cohort of a randomized controlled trial [11]. Therefore, it cannot be considered to be representative for the general transplant recipient population. Thirdly, as explained before, viral load was measured employing an in-house protocol, instead of the WHO standard. This makes the comparison with the viral load in other studies difficult. Fourthly, the study population, because of their low immunological

risk, was not at high risk for EBV-related complications. Accordingly, we observed relatively low replication values, despite the high incidence of reactivation. This limits the relevance of the conclusions on clinically relevant complications associated with EBV reactivation. Lastly, the follow-up time of the study does not allow for an evaluation of the incidence of PTLD, since most cases occur after the first post-transplantation year [38].

In spite of the aforementioned limitations, we have performed a comprehensive analysis of the risk factors and clinical complications of EBV reactivation in a large multi-centre cohort. Our analysis suggests pretransplant EBV shedding as an important risk factor associated with post-transplant EBV reactivation. Because of this, we hypothesize that EBV shedding could be employed as a biomarker for personalized immunosuppressive therapy. Furthermore, despite the association with decreased haemoglobin levels, EBV reactivations do not severely influence the post-transplant outcome in the short-term follow-up. An association between EBV and acute rejection can be explained by the diminished antiviral immune surveillance during anti-rejection immunosuppressive therapy. We consider that our results support the employed immunosuppressive regimes as relatively safe regarding EBV-associated complications. However, since

the incidence of PTLD increases with the transplant age, further follow-up studies are required to explore EBV pathogenicity in the long-term post-transplant course.

Acknowledgements

Conflict of interest

The authors have no conflicts of interest to declare.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Results of the prediction of EBV employing the marker subsets. Each row represents a marker subset, each column represents the EBV group employed for comparison (either EBV$^+$ or EBV$_{increased}$) and the visit of reference. The numbers and colour coding indicate the quality of achieved prediction, as estimated employing the BACC. NA values correspond to marker subsets for which not enough values were available for the EBV group to attempt prediction.

Figure S2. Forest plot comparing EBV$_{increased}$ versus EBV$^-$ patients for selected demographic factors, outcomes and transplant complications. The square denotes the odds ratio, whereas an odds ratio over 1 means an increased higher incidence among EBV$_{increased}$ patients; the grey lines denote the 95% confidence interval.

Figure S3. Expression of the co-inhibitory genes LAG3 and CD200 in the patient sub-cohorts two weeks post-transplant. The left column shows the comparison between EBV$^-$ and EBV$^+$, while the right column shows the comparison between EBV$^-$ and EBV$_{increased}$. Note that the shown P values are not adjusted for multiple testing, for the adjusted P values see Tables S1 and S2.

Table S1. Results for the association of all measured markers with EBV$^+$. The values of each measured marker for the EBV$^+$ and the EBV$^-$ patients are shown, as well as the P value of the difference and the adjusted P value for each sub-cohort.

Table S2. Results for the association of all measured markers with EBV$_{increased}$. The values of each measured marker for the EBV$_{increased}$ and the EBV$^-$ patients are shown, as well as the P value of the difference and adjusted P value for each sub-cohort.

Data S1. Detailed description of the methods employed for measuring the bio-marker panel and the statistical methods.

REFERENCES

1. Babcock GJ, Decker LL, Freeman RB, Thorley-Lawson DA. Epstein-Barr virus-infected resting memory B cells, not proliferating lymphoblasts, accumulate in the peripheral blood of immunosuppressed patients. *J Exp Med* 1999; **190**: 567–576. https://doi.org/10.1084/jem.190.4.567.

2. Nijland ML, Kersten MJ, Pals ST, Bemelman FJ, Ten Berge IJM. Epstein-Barr virus-positive posttransplant lymphoproliferative disease after solid organ transplantation: pathogenesis, clinical manifestations, diagnosis, and management. *Transplant Direct* 2016; **2**: e48. https://doi.org/10.1097/TXD.0000000000000557.

3. 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. https://doi.org/10.1111/ajt.12009.

4. Blazquez-Navarro A, Dang-Heine C, Wittenbrink N, et al. BKV, CMV, and EBV interactions and their effect on graft function one year post-renal transplantation: results from a large multi-centre study. *EBioMedicine* 2018; **34**: 113–121. https://doi.org/10.1016/j.ebiom.2018.07.017.

5. Rivera-Soto R, Damania B. Modulation of angiogenic processes by the Human Gammaherpesviruses, Epstein – Barr Virus and Kaposi's. *Front Immunol* 2019; **10**: 1544. https://doi.org/10.3389/fmicb.2019.01544.

6. Andrei G, Trompet E, Snoeck R. Novel therapeutics for Epstein – Barr Virus. *Molecules* 2019; **24**: 997. https://doi.org/10.3390/molecules24050997.

7. Ng S-B, Khoury JD. Epstein-Barr virus in lymphoproliferative processes: an update for the diagnostic pathologist. *Adv Anat Pathol* 2009; **16**: 40–55. https://doi.org/10.1097/PAP.0b013e3181916029.

8. Dierickx D, Habermann TM. Post-transplantation lymphoproliferative disorders in adults. *N Engl J Med* 2018; **378**: 549–562. https://doi.org/10.1056/NEJMra1702693.

9. Petrara MR, Giunco S, Serraino D, Dolcetti R, De Rossi A. Post-transplant lymphoproliferative disorders: From epidemiology to pathogenesis-driven treatment. *Cancer Lett* 2015; **369**: 37–44. https://doi.org/10.1016/j.canlet.2015.08.007.

10. Verghese PS, Schmeling DO, Filtz EA, Grimm JM, Matas AJ, Balfour HH. Transplantation of solid organ recipients shedding Epstein-Barr virus DNA pre-transplant: A prospective study. *Clin Transpl* 2017; **31**: e13116. https://doi.org/10.1111/ctr.13116.

11. Thomusch O, Wiesener M, Opgenoorth M, et al. Rabbit-ATG or basiliximab induction for rapid steroid withdrawal after renal transplantation

(Harmony): an open-label, multicentre, randomised controlled trial. *Lancet* 2016; **388**: 3006–3016. https://doi.org/10.1016/S0140-6736(16)32187-0.

12. Blazquez-Navarro A, Dang-Heine C, Bauer C, et al. Sex-associated differences in cytomegalovirus prevention: Prophylactic strategy is potentially associated with a strong kidney function impairment in female renal transplant patients. *Front Pharmacol* 2020; https://doi.org/10.3389/fphar.2020.534681.

13. Levey AS, Stevens LA, Schmid CH, et al. CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**: 604–612. https://doi.org/10.7326/0003-4819-150-9-200905050-00006.

14. Solez K, Colvin RB, Racusen LC, et al. Banff '05 meeting report: Differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant* 2007; **7**: 518–526. https://doi.org/10.1111/j.1600-6143.2006.01688.x.

15. Baron U, Werner J, Schildknecht K, et al. Epigenetic immune cell counting in human blood samples for immunodiagnostics. *Sci Transl Med* 2018; **10**: eaan3508. https://doi.org/10.1126/scitranslmed.aan3508.

16. Bajda S, Blazquez-Navarro A, Samans B, et al. The role of soluble mediators in the clinical course of EBV infection and B cell homeostasis after kidney transplantation. *Sci Rep* 2020; **10**: 1–13. https://doi.org/10.1038/s41598-020-76607-z.

17. Wittenbrink N, Herrmann S, Blazquez-Navarro A, et al. A novel approach reveals that HLA class 1 single antigen bead-signatures provide a means of high-accuracy pre-transplant risk assessment of acute cellular rejection. *BMC Immunol* 2019; **20**: 11. https://doi.org/10.1186/s12865-019-0291-2.

18. Liaw A, Wiener M. Classification and Regression by randomForest. *R News* 2002; **2**: 18–22.

19. Verghese PS, Schmeling DO, Knight JA, Matas AJ, Balfour HH Jr. The impact of donor viral replication at transplant on recipient infections posttransplant: a prospective study. *Transplantation* 2015; **99**: 602–608. https://doi.org/10.1097/TP.0000000000000354.

20. Ling PD, Lednicky JA, Keitel WA, et al. The dynamics of herpesvirus and polyomavirus reactivation and shedding in healthy adults: A 14-month longitudinal study. *J Infect Dis* 2003; **187**: 1571–1580. https://doi.org/10.1086/374739.

21. Johnson KH, Webb CH, Schmeling DO, Brundage RC, Balfour HH. Epstein-Barr virus dynamics in asymptomatic immunocompetent adults: An intensive 6-month study. *Clin Transl Immunol* 2016; **5**: e81–e85. https://doi.org/10.1038/cti.2016.28.

22. Zoccali C, Vanholder R, Massy ZA, et al. The systemic nature of CKD. *Nat Rev Nephrol* 2017; **13**: 344–358. https://doi.org/10.1038/nrneph.2017.52.

23. Sharif MR, Chitsazian Z, Moosavian M, et al. Immune disorders in hemodialysis patients. *Iran J Kidney Dis* 2015; **9**: 84–96.

24. Loginov R, Aalto S, Piiparinen H, et al. Monitoring of EBV-DNAemia by quantitative real-time PCR after adult liver transplantation. *J Clin Virol* 2006; **37**: 104–108. https://doi.org/10.1016/j.jcv.2006.06.012.

25. Morton M, Coupes B, Roberts SA, et al. Epstein-Barr virus infection in adult renal transplant recipients. *Am J Transplant* 2014; **14**: 1619–1629. https://doi.org/10.1111/ajt.12703.

26. 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. https://doi.org/10.1111/j.1399-0012.2012.01608.x.

27. Babel N, Schwarzmann F, Prang N, et al. Association between Epstein-Barr virus infection and late acute transplant rejection in long-term transplant patients. *Transplantation* 2001; **72**: 736–739. https://doi.org/10.1097/00007890-200108270-00031.

28. Rygiel TP, Karnam G, Goverse G, Van Der Marel APJ, Greuter MJ, Van Schaarenburg RA. CD200-CD200R signaling suppresses anti-tumor responses independently of CD200 expression on the tumor. *Oncogene* 2012; **31**: 2979–2988. https://doi.org/10.1038/onc.2011.477.

29. Qin S, Xu L, Yi M, Yu S, Wu K. Novel immune checkpoint targets: moving beyond PD-1 and CTLA-4. *Mol Cancer* 2019; **18**: 155–. https://doi.org/10.1186/s12943-019-1091-2.

30. Vaughan JW, Shi M, Horna P, Olteanu H. Increased CD200 expression in post-transplant lymphoproliferative disorders correlates with an increased frequency of FoxP3(+) regulatory T cells. *Ann Diagn Pathol* 2020; **48**: 151585. https://doi.org/10.1016/j.anndiagpath.2020.151585.

31. Panda A, Qian F, Mohanty S, et al. Age-associated decrease in TLR function in primary human dendritic cells predicts influenza vaccine response. *J Immunol* 2010; **184**: 2518–2527. https://doi.org/10.4049/jimmunol.0901022.

32. Ribas FS, Savassi-Ribas F, dos Santos G, et al. Impact assessment and investigation of factors associated with herpesviruses viremia in the first year of renal transplantation. *J Med Virol* 2019; **92**: 107–112. https://doi.org/10.1002/jmv.25580.

33. Ammatuna P, Campisi G, Giovannelli L, et al. Presence of Epstein-Barr virus, cytomegalovirus and human papillomavirus in normal oral mucosa of HIV-infected and renal transplant patients. *Oral Dis* 2001; **7**: 34–40.

34. de Santana Sarmento D, Tozetto-Mendoza T, Masami Sumita L, (2018). Oral shedding of human herpesviruses in renal transplant recipients. *Clinical Oral Investigations*, **22**: 885–891. http://dx.doi.org/10.1007/s00784-017-2166-3.

35. Velentgas P, Dreyer NA, Nourjah P, Smith SR, Torchia MM, eds. *Developing a Protocol for Observational Comparative Effectiveness Research A User's Guide*. Rockville, MD: Agency for Healthcare Research and Quality, 2013. https://www.ncbi.nlm.nih.gov/books/NBK126190/pdf/Bookshelf_NBK126190.pdf.

36. Bender R, Lange S. Adjusting for multiple testing - when and how? *J Clin Epidemiol* 2001; **54**: 343–349. https://doi.org/10.1016/S0895-4356(00)00314-0.

37. Li G, Taljaard M, Van den Heuvel ER, et al. An introduction to multiplicity issues in clinical trials: the what, why, when and how. *Int J Epidemiol* 2017; **46**: 746–755. https://doi.org/10.1093/ije/dyw320.

38. Végso G, Hajdu M, Sebestyén A. Lymphoproliferative disorders after solid organ transplantation-classification, incidence, risk factors, early detection and treatment options. *Pathol Oncol Res* 2011; **17**: 443–454. https://doi.org/10.1007/s12253-010-9329-8.