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Research Paper

BKV, CMV, and EBV Interactions and their Effect on Graft Function One Year Post-Renal Transplantation: Results from a Large Multi-Centre Study

Arturo Blazquez-Navarro [a,b], Chantip Dang-Heine [a], Nicole Wittenbrink [b], Chris Bauer [c], Kerstin Wolk [a,d], Robert Sabat [d,e], Timm H. Westhoff [f], Birgit Sawitzki [a,j], Petra Reinke [a,g], Oliver Thomusch [h], Christian Hugo [i], Michal Or-Guil [b,*,1], Nina Babel [a,f,**,1]

[a] Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany
[b] Systems Immunology Lab, Department of Biology, Humboldt-Universität zu Berlin, Philippstr. 13, 10115 Berlin, Germany
[c] MicroDiscovery GmbH, Marienburger Str. 1, 10405 Berlin, Germany
[d] Psoriasis Research and Treatment Center, Institute of Medical Immunology, Department of Dermatology and Allergy, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany
[e] Interdisciplinary Group of Molecular Immunopathology, Institute of Medical Immunology, Department of Dermatology and Allergy, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany.
[f] Medical Department 1, Universitätsklinikum der Ruhr-Universität Bochum, Ruhr-Universität Bochum, Hölkeskampring 40, 44625 Herne, Germany
[g] Berlin Center for Advanced Therapies (BeCAT), Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany.
[h] Klinik für Allgemein- und Viszeralchirurgie, Universitätsklinikum Freiburg, Hugstetter Straße 55, 79106 Freiburg, Germany
[i] Universitätsklinikum Carl Gustav Carus, Medizinische Klinik III - Bereich Nephrologie, Fetscherstraße 74, 01307 Dresden, Germany
[j] Molecular Immune Modulation, Institute for Medical Immunology, Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

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ABSTRACT

Background: BK virus (BKV), Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) reactivations are common after kidney transplantation and associated with increased morbidity and mortality. Although CMV might be a risk factor for BKV and EBV, the effects of combined reactivations remain unknown. The purpose of this study is to ascertain the interaction and effects on graft function of these reactivations.
Methods: 3715 serum samples from 540 kidney transplant recipients were analysed for viral load by qPCR. Measurements were performed throughout eight visits during the first post-transplantation year. Clinical characteristics, including graft function (GFR), were collected in parallel.
Findings: BKV had the highest prevalence and viral loads. BKV or CMV viral loads over 10,000 copies·mL^{-1} led to significant GFR impairment. 57 patients had BKV-CMV combined reactivation, both reactivations were significantly associated ($p = 0{\cdot}005$). Combined reactivation was associated with a significant GFR reduction one year post-transplantation of $11{\cdot}7$ mL·min^{-1}·$1{\cdot}73$ m^{-2} ($p = 0{\cdot}02$) at relatively low thresholds (BKV > 1000 and CMV > 4000 copies·mL^{-1}). For EBV, a significant association was found with CMV reactivation ($p = 0{\cdot}02$), but no GFR reduction was found. Long cold ischaemia times were a further risk factor for high CMV load.
Interpretation: BKV-CMV combined reactivation has a deep impact on renal function one year post-transplantation and therefore most likely on long-term allograft function, even at low viral loads. Frequent viral monitoring and subsequent interventions for low BKV and/or CMV viraemia levels and/or long cold ischaemia time are recommended.
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* Correspondence to: Michal Or-Guil, Systems Immunology Lab, Department of Biology, Humboldt-Universität zu Berlin, Philippstr. 13, 10115 Berlin, Germany
** Correpondence to: Nina Babel, Medical Department 1, Universitätsklinikum der Ruhr-Universität Bochum, Ruhr-Universität Bochum, Hölkeskampring 40, 44625 Herne, Germany
E-mail addresses: m.orguil@biologie.hu-berlin.de (M. Or-Guil), nina.babel@charite.de (N. Babel).
[1] Corresponding authors contributed equally to this work.

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Research in context

Evidence Before this Study

Viral reactivations of BK virus (BKV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV) are common complications in recipients of renal transplantation. Combined reactivations of these viruses have been observed repeatedly in the past and interplay between BKV and CMV has been shown *in vitro*. Different interaction mechanisms have been proposed. However, it is currently unclear whether there are associations in viral reactivations *in vivo*. Moreover, it is not clear so far what is the cause of such combined reactivations and whether combined reactivations have more serious impact on graft function than the corresponding mono-reactivations. To obtain information on the state-of-art, we searched MEDLINE, PubMed, and Google Scholar for papers published after January 2003, using the terms "renal transplantation BKV", "renal transplantation CMV", "renal transplantation EBV", "coinfection BKV CMV", "coinfection BKV EBV", "coinfection CMV EBV". No language restrictions were employed. The quality of evidence was assessed prioritizing epidemiological studies over case reports and *in vitro* studies.Added Value of this Study

This is the first large, prospective multi-centre study to systematically analyse the clinical course of BKV, CMV, and EBV reactivations at eight pre-defined time points during the first post-transplantation year. Almost ten thousand viral load measurements were performed. It is the first study to provide clinical evidence of the relevance of BKV-CMV combined reactivations, showing, already at moderate viral loads (BKV > 1000 and CMV > 4000 copies·mL^{-1}), an impact on renal function one year post-transplantation with a median drop in renal function of 11·7 mL·min^{-1}·1·73 m^{-2}. This observation is reinforced by the fact that a significant association was found between BKV and CMV during the first post-transplantation year. Moreover, it is the first large study to find an association between cold ischaemia time and high level CMV viral load: High-level CMV (>10,000 copies·mL^{-1}) was associated with significantly longer cold ischaemia time for cadaveric graft (median difference: 284 min), compared to patients without CMV or CMV below the threshold. Furthermore, this study shows BKV as the most relevant viral adverse event in kidney transplantation, as it had the highest prevalence, the highest viral loads and lowest clearing rate. Our results have revealed a prevalence of presumptive BKV nephropathy of 10·9% (over the 1–10% prevalence in the literature), in spite of the patients belonging to an immunological low-risk cohort. In conclusion, it is a confirmation that BKV is an emergent pathogen that must be tackled in order to improve the efficacy of current transplantation protocols.Implications of All the Available Evidence

We have provided the most systematic analysis so far of BKV, CMV, and EBV virus reactivations in renal transplantation, as part of a large, prospective multi-centre study. Their viral loads were analysed at eight time points during the first transplantation year. With our results, we showed a clinical impact of BKV-CMV combined reactivation, even at low viral load levels. In addition, we performed in-depth analyses of the impact of different modifiable and non-modifiable risk factors on virus reactivation. Therefore, we consider our work as crucial for the management of viral reactivations after kidney transplantation, leading to a better monitoring and treatment for kidney transplantation patients with BKV and/or CMV low viral loads, as well as patients with long cold ischaemia times and additional CMV risk factors.

1. Introduction

Viral reactivations are a major cause of morbidity and mortality for recipients of solid organ transplantation [1]. In kidney transplantation, BK virus (BKV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV) are major pathogens. These viruses are very common in healthy population, with an approximate prevalence of 80%, 60%, and 90%, respectively [2–4]. Primary infection usually occurs during childhood, but the virus stays latent and asymptomatic under normal conditions [5,6]. Individuals with compromised immune systems, i.e. after a solid organ transplantation, are prone to both primary infection and reactivations with clinically relevant symptoms [7,8].

BKV is an emerging pathogen and the cause of BKV-associated nephropathy (BKVAN), a major complication in renal transplantation [6]. It is linked to kidney malfunction and rejection, leading to graft loss in up to 60% of affected patients [6,8,9]. The incidence of BKVAN is 1–10% in renal transplantation [10]. BKVAN is usually encountered in a context of over-immunosuppression, even though it is not associated with a specific immunosuppressive drug [9,11,12]. Early diagnosis is vital for a successful treatment, but BKVAN progression occurs without clinical signs except for increasing serum creatinine concentrations and diagnosis relies on renal biopsy [9,11]. However, BKV serum load over 10,000 copies·mL^{-1} is a generally accepted surrogate marker defining "presumptive BKVAN" [11].

CMV is a major viral pathogen after kidney transplantation, linked among others to retinitis, pneumonitis, colitis, encephalitis and importantly, allograft damage, allograft loss and death [5,8,13,14]. CMV proliferation may occur through reactivation of a latent infection, a new donor-transmitted infection or acquired from the general population due to the immunosuppression [13]. However, the highest risk is encountered by CMV seronegative patients receiving a transplant from a seropositive donor (D^+R^-) [13]. EBV in kidney transplantation is mainly associated with post-transplant lymphoproliferative disorders (PTLD) [5,7]. PTLD is a severe complication in solid organ transplantation, occurring in around 1% of patients mostly after the first post-transplant year [7,15,16]. It comprises a very broad spectrum of disorders, from spontaneously regressing to lethal B cell proliferations [4,7].

In this work, we assess the impact and relevance of BKV, CMV, and EBV reactivations in a large, prospective multi-centre study, analysing renal transplant in clinical follow-up during the first year after transplantation. Our work focuses on potential interactions between viruses and their combined impact on graft function, as well as the risk factors associated with each virus, including the role of immunosuppressive therapy.

2. Patients and Methods

2.1. Patient Population

We conducted a sub-study within the randomized, multi-centre, investigator-initiated Harmony trial (NCT 00724022) [17] to prospectively monitor viral load of BKV, CMV, and EBV at predetermined eight study visits and correlate it with clinical outcome parameters. Following the KDIGO clinical guideline, BKV viral load monitoring was performed in serum rather than urine, as the former has a higher BKVAN diagnostic value [18,19]. Viral monitoring was non-interventional and centrally performed. The study was carried out in compliance with the Declaration of Helsinki and Good Clinical Practice. A total of 540 patients undergoing kidney transplantation between 08/2008 and 11/2012 were analysed (Fig. 1).

2.2. Patient Medication

Patients were randomized to one of three therapeutic groups, as described before [17]. The immunosuppressive therapy included induction with either monoclonal IL-2R antibody basiliximab (arms A and

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