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Risk Factors for BK Virus Infection after Kidney Transplantation, London, Ontario 2016

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in
Epidemiology and Biostatistics

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ABSTRACT

Our objective was to determine the risk factors for BK virus infection in renal allograft recipients in the first year after transplantation. In this cohort, we included all patients who received renal allograft at London Health Sciences Centre (LHSC) between 2012 and 2014. We continued post-transplantation follow-up for one year. Of 175 patients (37% female) with median age (range) of 53 (14-82) years, 40 (22.9%) developed BK viremia (median interval: 100 days, range: 35-264). Recipient age, recipient gender, hemodialysis (HD) vs peritoneal dialysis (PD), Human Leukocyte Antigens A1, B35 and Cw4 increased the risk of post-transplant BKV infection. However, donor gender, donor age, deceased vs living donor, delayed graft function, ABO incompatibility and retransplantation did not increase the risk. PD and HD patients do not appear to have equal risks at the time of transplantation. Further studies are required to determine the immunologic reasons for this difference.

KEYWORDS: Polyomavirus, Bk Virus, Viremia, Kidney Transplantation

CO-AUTHORSHIP STATEMENT

The study described here was designed and executed by Dr Seyed M Hosseini-Moghaddam. This includes but is not limited to study conception, data analysis and interpretation. J Kum, H Alharbi, and G Singh contributed in data collection. Dr Q Xu provided data related to HLA typing. Regular feedback was received by Thesis Advisory Committee. The manuscript was primarily authored by Dr Seyed M Hosseini-Moghaddam.

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LIST OF ABBREVIATIONS

ABOi	ABO incompatible
Alemtuzumab	A monoclonal antibody binds to CD52, a lymphocyte protein
AMI	Antibody mediated immunity
AUC	Area under curve
AZA	Azathioprine
BKV	BK polyomavirus
Cmax	Maximum concentration of the medication in blood
CMI	Cell mediated immunity
CNI	Calcineurin inhibitors including tacrolimus and cyclosporine
CNS	Central Nervous System
CTL	Cytotoxic T Lymphocyte
FDA	Food and Drug Administration
CyA	Cyclosporine A
DDI	donor-derived infection
DSA	Donor specific antibody
ELISPOT	The Enzyme-Linked ImmunoSpot
HLA	Human Leukocyte Antigen
ESRD	End-stage renal disease

HD	Hemodialysis
JCV	Polyomavirus JC
MCP-1	Monocyte chemoattractant protein-1
MHC	Major histocompatibility complex
OKT3	Muromonab-CD3
PD	Peritoneal dialysis
IVIG	intravenous immune globulin
LDK	Living kidney donor
MMF	Mycophenolate mofetil or myfortic
mTOR inhibitors	Mammalian target of rapamycin, sirolimus and everolimus
PML	Progressive multifocal leukoencephalopathy
Preemptive transplantation	Patients who receive renal transplantation prior to requirement for dialysis
PyVAN	Polyomavirus associated nephropathy
RCT	Randomized clinical trial
rATG	Rabbit antithymocyte globulin
TAC	Tacrolimus
Viremia	Bloodstream infection with viruses
Viruria	Urine infection with viruses

CHAPTER 1: INTRODUCTION

BKV is a member of the polyomavirus family. It was initially isolated in the urine of a renal transplant recipient with ureteral stenosis in 1971 and was named after the patient's initials 'B.K.'(1). Further studies showed infection with this virus may cause significant tissue changes in the renal allograft and subsequent graft loss(2). Infection with this virus begins with viral replication and shedding in the urine (viruria) which consequently progresses to blood stream infection (viremia) and eventually to polyomavirus associated nephropathy (PyVAN)(3). The frequency of viruria in renal transplant recipients is around 30–40% while 10– 15% of recipients develop BK viremia, and only 2–5% develop PyVAN(4). The ranges in these figures may be related to variations in risk factors, screening strategies, and immunosuppressive regimens. Regardless, BKV nephropathy is a known cause of allograft loss in kidney transplant recipients.

Previous investigations evaluating risk factors for BKV infection and PyVAN have demonstrated inconsistent findings(5-6-7). Studies of recipient pairs who received kidney allografts from the same donor demonstrated that BKV infection can be a donor-derived infection (DDI)(8,9). In patients with donor origin BKV infection, early testing of urine samples from the recipients was helpful to identify those at risk for BKV-associated nephropathy(10). Recent data supported this hypothesis by demonstrating the transmission of the same genotype of BKV from the donors to the recipients (11). BKV infection can also arise as a reactivated virus infection with recipient origin(12). Thus, the source of post-transplant BKV infection could be either the donor or the recipient.

Previous studies identified risk factors of BKV infection including donor characteristics (female gender, African-American ethnicity, deceased donor vs living donor, ischemic-reperfusion injury, elevated BKV-specific antibody level as evidence of recent infection, Human Leukocyte Antigen (HLA) mismatches, recipient characteristics (male gender, age older than 46 years, poor BKV-specific T-cell activity) and post-transplant variables (acute rejection and antirejection immunosuppressive therapy, ABO desensitization, cumulative doses of steroid, lymphocyte depleting antibodies, higher blood levels of immunosuppressive medications, tacrolimus vs cyclosporine, mycophenolate mofetil) (5·6·13·14·15·16·13·17·18·19)

In this cohort study, we determined the risk factors associated with BKV infection in renal transplant recipients. These risk factors include the variables related to the donor, recipient, allograft and post-transplant immunosuppression. We studied the association of the following variables with BKV infection in univariable and multivariable analysis: donor age, donor gender, recipient age, recipient gender, pre-transplant renal replacement therapy, HLA typing, HLA mismatch, cold ischemia time, cytomegalovirus serostatus at the time of transplantation, delayed graft function, donor status (living or deceased), rejection, ABO incompatibility, maintenance immunosuppression, and tacrolimus blood level.

The remainder of this thesis is organized as follows: Chapter 2 is a review of the peer-reviewed literature on BK virus and PyVAN; Chapter 3 contains a manuscript intended for publication in a peer-reviewed journal, and Chapter 4 is a conclusion and general discussion of the work undertaken as part of this thesis. The Research Ethics Board approval can be found in Appendix A. Appendix B contains supplementary material for the manuscript in Chapter 3.

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CHAPTER 2: LITERATURE REVIEW

General Introduction to Polyomaviruses

The human polyomaviruses, mainly JC virus (JCV) and BK virus (BKV), are ubiquitous in most human populations throughout the world. Polyomaviruses can cause invasive infection. In immunocompromised patients, JCV infection causes a demyelinating disease of the central nervous system (CNS) and progressive multifocal leukoencephalopathy (PML)⁽¹⁾ while BKV causes polyomavirus associated nephropathy (PyVAN) and ureteral stenosis in renal transplant recipients. BKV and JCV can both cause hemorrhagic cystitis in stem cell transplant patients⁽²⁾. PML is an opportunistic infection in acquired immunodeficiency syndrome (AIDS) in HIV- positive patients. New immunosuppressive treatments for non-transplant patients have also been shown to be associated with polyomavirus infection. Natalizumab is one of the new monoclonal antibodies that has been shown to be effective in the management of multiple sclerosis and Crohn's disease. So far, more than 400 cases of natalizumab-related PML have been reported worldwide. ⁽³⁾.

Recently, nine new human polyomaviruses have been isolated. These new members of the genus were mainly named based on the site of discovery, their geographic origins, the diseases that they cause, or order of discovery: MWPyV (Malawi)⁽⁴⁾, WUPyV (Washington University)⁽⁵⁾, and KIPyV (Karolinska Institute)⁽⁶⁾, STLPyV (St Louis), MCPyV (Merkel cell carcinoma)⁽⁷⁾, TSPyV (trichodysplasia spinulosa)⁽⁸⁾; HPyV6, HPyV7, and HPyV9 (human polyomaviruses 6, 7, and 9)^(9,10). MCPyV is currently known as the cause of Merkel cell carcinoma, a cutaneous cancer that can involve both immunocompetent individuals and immunocompromised patients such as renal transplant recipients⁽⁷⁾ .

BK Virus

Previous studies showed as much as 85% of the general adult population are seropositive for BKV(11). The prevalence of BKV infection is initially low in children; however, the seroprevalence increases with age(11). Thus, elderly people are more likely to be seropositive than children, adolescents and adults. This discrepancy of seroprevalence and the frequency of BKV infection in different age groups are important in understanding the epidemiology of BKV infection.

BKV does not usually cause a disease in immunocompetent individuals. However, infection with this virus is associated with several diseases in immunocompromised individuals including ureteral stenosis(12) and PyVAN(13) in renal transplant recipients and hemorrhagic cystitis(14) in stem cell transplant patients.

BKV replicates in the urothelial cells and the rate of replication increases with immunosuppression. Approximately 15-35% of renal transplant recipients develop BK viruria(15). Detection of viruria before viremia is clear evidence of replication of the virus in the urinary tract system. However, the urinary viral load is not similar in all renal transplant patients, and only patients with a high level of viruria develop viremia. One third of recipients with a high level of BK viruria ($4 \log_{10}$ genome equivalents (geq)/mL) develop BK viremia which is linked to polyomavirus associated nephropathy (PyVAN)(16). Since most current data is limited to immunocompromised patients, our understanding of the epidemiology of this virus is still incomplete.

Primary Infection and Transmission

Primary infection with BKV occurs in childhood as evidenced by increasing BKV seroprevalence in the first decade of life(11). Natural BKV transmission is likely to occur via the respiratory or oral routes(17). After primary infection, BKV can be detected in renal tubular epithelial cells, where they may remain latent.

Respiratory and gastrointestinal (GI) transmission of BK virus has been confirmed in animal models. However, GI transmission was delayed and not as effective as the respiratory model(18). BKV was also detected in oral fluids(17). BKV infection and replication of the virus was shown in salivary gland cells(17). These data suggested the potential oral route of transmission for BKV.

In a study, stool samples and rectal swabs were collected from hospitalized pediatric patients. This study showed polyomaviruses are frequently detectable in stool samples. This finding demonstrates that fecal-oral transmission of BKV may also play a role(19).

Some data suggest vertical transmission of BK from mother to newborn either during pregnancy or after delivery(20). The frequency of BK viraemia increases to around 35% during pregnancy suggesting the risk of vertical transmission(21). Animal data also showed vertical transmission of polyomaviruses(22:23). BK virus can cross the placenta and become latent in fetal organs(24). Recent data showed probable vertical transmission of BKV in human(25). On the other hand, population-based studies showed the same genotypes of BKV in urine samples collected from Japanese-Americans and from other southern Californians in Los Angeles. Since this genotype (subtype I subgroup Ib-2) is rare in native Japanese, this data suggest transmission outside of the family is common(26).

Immunology

The immune system plays an essential role in the control of BKV replication and resolution of PyVAN. CD4⁺ and CD8⁺ T cells both are main components of cell mediated immunity to control BKV. T-cell response targets both nonstructural and viral capsid proteins and this response can be measured via ELISPOT and tetramer staining(27). Cytotoxic T-cells sensitized to BK virus antigens were detected in healthy volunteers and renal transplants(28). In kidney transplant recipients, a considerable CD8 response was associated with lower BK viral loads in blood and urine, while poor response was associated with high BK titers and viral persistence(29). Thus, reduction of immunosuppression appears to be associated with decreases in viruria and viremia.

Cell mediated immunity plays a pivotal role in controlling BKV infection. Cytotoxic T cells (CTL) kill the cells infected with BKV after recognition of damaged segments of viral DNA(30). This finding helped researchers develop cellular immunotherapy for the management of BKV infection and PyVAN. Ex vivo reactivation of BKV-specific CTL was developed from BKV-seropositive healthy donors and renal transplant recipients through stimulation with dendritic cells (antigen presenting cells) pulsed with inactivated BKV. Those cytotoxic T cell lines demonstrated BKV specificity, as an efficient lysis of BKV-infected targets(31).

Infected dendritic cells may induce CTLs(32). Interestingly, the BKV CTL epitopes bear remarkable homology with the CTL epitopes of JCV(29). The same population of T-cells are probably effective against these two closely related viruses. However, it is not clear why suppression of CMI after transplantation causes replication of BKV but not JCV. (27).

Different immunosuppressive regimens have been shown to be associated with BKV infection and PyVAN. Receiving OKT3, rATG and alemtuzumab, tacrolimus and MMF significantly increase the risk of BKV infection(33-34). It appears that the role of these immunosuppressive medications is mainly through decreasing the function of CMI.

The role of antibody mediated immunity was also demonstrated in polyomavirus infection. Treatment with immunoglobulins can control the BKV infection and PyVAN(35-36-37). On the other hand, treatment of acute rejection with high doses of immunoglobulin was associated with increased BK viral load and worsening of PyVAN(38). Patients with PyVAN showed the highest frequencies of BKV-specific T cells at time of recovery, the highest rise in BKV-specific IgG and persistence of enhanced IgM levels(39). These studies provided excellent evidence to support the roles of CMI and AMI to control BKV infection.

Allograft Rejection

Acute rejection is an independent risk factor for BK virus infection after transplantation(33,40). Cell-mediated, antibody-mediated or steroid resistant rejections all increase the risk of BKV infection and PyVAN(33). The effect of allograft rejection may be related to the treatment of rejection with immunosuppressive medication and not necessarily rejection itself(41).

Blood Groups and ABO-incompatibility

When blood groups of donor and recipient are not matched (ABOi or ABO incompatibility), the recipient is at a risk of allograft rejection. Generally, recipients with blood group O and B are at a higher risk of rejection. Patients with ABOi should receive medical treatment and immunosuppression before and after transplantation. This treatment is called desensitization which includes removing antibodies from blood circulation (plasmapheresis), spleen removal (splenectomy), intravenous immunoglobulin, and immunosuppression.

It has been demonstrated that ABO-incompatible kidney recipients are at greater risk for BKVAN than HLA-incompatible kidney recipients (42). However, it is not clear that ABOi itself increases the risk or whether a higher incidence of BKV infection and PyVAN in ABOi patients is related to desensitization. ABOi renal transplants are more likely to develop CMV infection, BKV-associated nephropathy, and even severe sepsis. T-cell depletion due to desensitization may increase the risk of T-cell-dependent infectious diseases. Elimination of B cells serving as antigen-presenting cells, thus causing impaired T-cell activation, will affect alloreactive T-cell activation and increases the chance of infection(43).

A study which included 26 ABOi renal transplant recipients and continued follow-up for one year after transplantation demonstrated a higher risk of BKV infection in patients receiving desensitization(44). In that study, patients received an intensified desensitization with rATG which was associated with significant immunosuppression. To resolve this risk, a study suggested using everolimus rather than mycophenolic acid in immunosuppression to decrease the risk of BKV infection(45).

A study from John Hopkins Hospital compared the incidence of BKV infection between 62 ABOi and 221 HLA incompatible patients. That study showed the risk for BKV infection is greater among ABOi than HLA-incompatible patients (17.7% vs. 5.9%)(42). This is the only study that demonstrated a significant association between ABOi and BKV infection and compared by HLA incompatibility.

HLA Antigens and BKV Infection

HLA or Major Histocompatibility Complex (MHC) are groups of genes in animals and humans that encode a variety of cell surface markers, antigen-presenting molecules, and other proteins involved in immune function. The HLA system is divided into 3 regions: class I, class II, and class III. The class I region contains the genes encoding the “classical” class I HLA antigens: HLA-A, B, and C.

Except for red blood cells and platelets, all human body cells have HLA class I. The class II region contains the genes that encode the HLA class II molecules including HLA-DP, DQ, and DR. Class II antigens are expressed on B cells, dendritic cells, and monocytes but can be induced during inflammation on many other cell types that normally have no expression.

The following facts explain the complexity of the HLA system in our body:

1. HLA class I contains an alpha chain and a beta chain. The alpha chain is coded for by genes within the MHC in chromosome 6; however, the beta chain, beta-2 microglobulin, is encoded on chromosome 15 and not in the MHC.
2. Each class contains several gene loci. Some HLA loci are very polymorphic; for example, over 4300 alleles are known for HLA-B and over 1900 alleles for HLA-DRB1.

3. We have polymorphic alpha and beta chains in DQ and DP antigens, which can make various combinations.

4. By contrast, DR dimers have an essentially invariant alpha chain, but extreme polymorphic beta chain.

5. The number of DR genes varies among people.

All these factors demonstrate the likelihood of interaction between HLA variables. The role of HLA in BKV infection has been evaluated in several investigations. Renal transplant patients may excrete some HLAs in their urine. Excretion of some HLAs in urine was shown before detection of allograft damage. Increased excretion of cells with HLA-DR surface antigens was associated with intragraft tubulointerstitial inflammation in patients with PyVAN(46). This suggests the role(s) of HLA molecules in inflammatory changes of the allograft after BKV infection.

Donor-recipient mismatching of HLA-B and HLA-DR may increase the risk of BKV infection (33). On the other hand, donor-recipient matching of HLA-A2, HLA-B44 and HLA-DR15 were shown to be associated with a lower risk of BKV infection(47). It has been recently shown recipient age younger than 18 years, male sex, HLA A, B, DR mismatches of 4 or greater, acute rejection, and the use of depleting antibody induction were main risk factors for BKV infection(48). This study showed the risk of BKV infection increases with rising of HLA mismatches. The number of HLA mismatches seems to be the strongest independent predictors of BK viremia(49,40). Recent data interestingly showed that the absence of HLA C7 increases the risk of sustained viremia(49). HLA C7 seems to provide a protective role against BKV infection. On the other hand, patients with ESRD secondary to diabetes and patients treated with sirolimus had lower odds of BKV infection(48).

BK Viruria and Viremia

Up to 20% of asymptomatic immunocompetent individuals may develop BK viruria(50), but viral shedding in the urine is higher in renal allograft recipients (57%) which correlates with the degrees of immunosuppression(51). In the urine of patients who are HIV positive, BK viral load increases with decreasing CD4+ T-cell counts(52). Although BKV is usually not detected in the peripheral blood of immunocompetent patients, the detection of BKV DNA in the plasma of renal transplant patients is an indication of development of PyVAN(53). The risk for viremia is higher in patients with viruria (particularly for viral load (VL) >4 log/mL) treated with tacrolimus(51). BK viremia regardless of allograft function is considered as presumptive BK nephropathy(53). A BK viral load more than 4 log copies/mL has been shown to be strongly associated with biopsy-proven PyVAN (16).

Immunosuppression

Previous studies assumed association of different immunosuppressive regimens with BKV infection. Consequently, the researchers used heterogeneous methods of immunosuppression reduction associated with a wide range of dose change (54:55). Most previous studies which showed effectiveness of immunosuppression reduction did not have control groups(56:54:57:55) or their sample sizes were small(57:58). Immunosuppression appears to be the most important risk factor for BKV infection (12). In a RCT, 200 adult renal transplants were followed for one year after transplantation. Of these, 23 patients (11.5%) developed BK viremia. The protocol of immunosuppression reduction included discontinuation of AZA or MMF. In case of failure to clear viremia in 4 weeks, the doses of CNIs were tapered. Using this approach, 95% of patients were able to clear viremia but 10 cases of rejection occurred (59).

To demonstrate which immunosuppressive medications are more associated with BKV infection, researchers began immunosuppression reduction with CNIs rather than MMF or AZA. In a cohort of 38 patients, decreasing immunosuppression was evaluated in three stages a) decreasing the dose of tacrolimus to achieve the trough level of 6-8ng/ml then b) further dose reduction of tacrolimus (targeting trough level of 4-6 ng/ml and eventually c) decreasing the dose of MMF by 50%. This approach was associated with 92% of clearance of viremia and 8.6% frequency of rejection(56). This study had no control group. This study supported the association between tacrolimus blood level and BKV infection.

In an uncontrolled cohort of 62 patients, progressive decreasing dose of tacrolimus targeting the level of 5–8 ng/mL as well as decreasing the dose of MMF targeting 600 mg/m²/day was associated with 100% clearance of viremia (16). This study showed probable effects of tacrolimus blood level and MMF in the pathogenesis of BKV infection and PyVAN.

Some centers preferred cessation of immunosuppressive medications rather than dose reduction. In a retrospective analysis, withdrawal of an immunosuppressive medication (mostly CNIs) in 17 patients was compared by reduction of immunosuppression (mostly MMF) in 18 patients. Withdrawal of immunosuppressive medications was significantly associated with better graft survival (one-year graft survival of 87.8% versus 56.2%, $P = 0.03$). Heterogeneous intervention, retrospective analysis and low number of patients were main limitations of this study(54). Unfortunately, this study did not show which components of immunosuppression are more likely associated with BKV infection and PyVAN. Enhancement of graft function was likely secondary to improvement of BK viremia.

Unfortunately, immunosuppression reduction may not be necessarily associated with good outcomes. In a cohort of 11 patients with BK viremia, this approach was associated with rejection in 3 patients and one graft loss(57). This study showed decreasing immunosuppression may not be a safe approach and may increase the chance of graft loss.

A recent study from China showed excellent effect of immunosuppression reduction. In this study, patients with presumptive PyVAN (BKV infection) were treated with 30-50% reduction in doses of tacrolimus and/or mycophenolate mofetil and were monitored for BKV every 3-6 months. All patients were followed for 5 years. Overall, 5-year patient and graft survival rates were 95.6% and 92.1%, respectively(55). The protocol for immunosuppression was quite heterogeneous with a wide range of immunosuppression reduction. This study did not have a control group. Their findings not only showed the effect of the doses of immunosuppressive medications in BKV infection and PyVAN. Interestingly, with reducing the magnitude of the risk factor (doses of immunosuppressive medications) the outcome (PyVAN) was reversible and this effect remained.

Some studies compared reduction of immunosuppression with medical treatment of BKV infection and PyVAN. In one study, 14 renal transplants with significant BK viremia received ciprofloxacin plus intravenous immunoglobulin. The control group (19 recipients) was treated with immunosuppression reduction. One-year graft survival and the rate of rejection were similar(58). This study did not have enough power to show the risk of rejection due to a small number of patients in each group. The findings of this study showed reducing of immunosuppression may reverse PyVAN although other interventions such as intravenous immunoglobulin and fluoroquinolones affected the results.

Early detection of sustained BKV-viremia is probably the key factor for the achievement of immunosuppression reduction approaches. Undeniably, all studies that reported favorable outcomes of immunosuppression reduction detected BKV-viremia early post-transplant, allowing for appropriate therapeutic intervention(60:57-59). However, if the diagnosis of BKV infection and PyVAN are made at a later stage, reduction of immunosuppression is likely to be less successful, probably due to more progressive irreversible injury of the renal allograft(54). Overall, these findings support the association between immunosuppression and BKV infection but also demonstrate reversibility of the outcome when we decrease the dose of the risk factor.

Medications with Anti-BKV Effects

Cidofovir is an antiviral medication that may be used for treatment of other viral infections such as CMV(61). This medication is an acyclic nucleoside phosphonate that acts as an inhibitor of DNA synthesis. It inhibits replication of BKV(62). Brincidofovir (BCV) is an oral form of this medication. BCV is ether-lipid ester conjugated prodrug of cidofovir. The lipid moiety of BCV is important for its pharmacokinetic properties, facilitating rapid uptake by cells and allowing oral administration(63). Recently, some clinical trials were carried out to show the efficacy of this medication against CMV infection. Expectedly, patients who were included in those studies did not develop BKV infection(64). The effect of BCV on CMV is related to inhibition of DNA polymerase but BKV utilizes host cell DNA polymerase and the anti-BKV mechanism of BCV is not clear. It is important to consider the effects of these medications in the risk analysis for BKV infection and PyVAN.

Leflunomide is an anti-metabolite, antirheumatic disease modifying agent. Leflunomide inhibits pyrimidine synthesis resulting in anti-proliferative and anti-inflammatory effects. Its metabolite, teriflunomide (A77 1726) has been found to reduce or stop the replication of BK in vitro and in animal models(65). Although the data related to efficacy of this medication showed quite variable findings, patients who are on this medication theoretically may be at a low risk of BKV infection.

Sirolimus and everolimus are two medications belonging to the family of mTOR inhibitors. Although they are immunosuppressive medications , they inhibit BKV replication in urothelial cells(66· 67).

As mentioned above, intravenous immune globulin (IVIG) may control BKV infection(35·68· 69). This medication is a pooled product which contains antibodies against different pathogens. It also contains antibodies against HLA antigens which inhibit rejection. This medication also has some immunomodulatory effects.

In conclusion, the human polyomaviruses have been shown to be common in human populations since their discovery in the 1950s. Many donor and recipient characteristics have been shown to have protective or risk effects in the context of BK virus infection and kidney transplantation. The next chapter of this thesis is a report of an original cohort study undertaken to add to our understanding of these risk and protective factors.

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CHAPTER 3¹

THE EFFECT OF PRETRANSPLANT HEMODIALYSIS VS. PERITONEAL DIALYSIS ON BK VIRUS INFECTION AFTER RENAL TRANSPLANTATION

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Abbreviations

ABOi: ABO incompatible, BKV: BK virus, CMI: Cell mediated immunity, CNI: Calcineurin inhibitors, DCD: Donation after cardiac death, DDI: donor-derived infection, ESRD: End-stage renal disease, HD: Hemodialysis, HLA: Human Leukocyte Antigen, IVIG: intravenous immune globulin, LD: Living donor, mTOR: mammalian target of rapamycin, NDD: Donation after neurological determination of death, OPT: Organ Procurement Transplant Network, PCR: polymerase chain reaction, PD: Peritoneal dialysis, PE: preemptive transplantation, PyVAN: Polyomavirus associated nephropathy, rATG: Rabbit antithymocyte globulin

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Introduction

BK virus is a polyomavirus which was initially isolated in the urine of a renal transplant recipient with ureteral stenosis (1,2). It was subsequently shown that BKV infection begins with replication in urothelial cells (3). Viral replication and shedding in the urine (viruria) consequently progresses to blood stream infection (viremia) and eventually to polyomavirus associated nephropathy (PyVAN) (4). The frequency of viruria in renal transplant recipients is estimated to be 30–40% while 10–15% of recipients develop BK viremia, and 2–5% develop PyVAN(5). The difference in the reported incidence rates seems to be related to risk factors, magnitude of exposure and screening strategies(6). BKV nephropathy is a considerable cause of allograft loss in kidney transplant recipients(7).

Previous investigations evaluating risk factors for BKV infection and PyVAN have demonstrated inconsistent findings(8-9-10). Some studies demonstrated that BKV infection can be a donor-derived infection (DDI) (11,12). Recent data demonstrated the same genotype of BKV in donors and recipients(13). Early post-transplant urine PCR (polymerase chain reaction) can detect BK viruria in recipients with donor-derived BKV infection (14). On the other hand, BKV infection can also represent a reactivated infection with recipient origin(15).

Previous studies showed probable risk factors of BKV infection including donor characteristics (female gender, deceased donor, ischemia-reperfusion injury, elevated BKV-specific antibody level, HLA-mismatches), recipient characteristics (male gender, age, poor BKV-specific T-cell activity) and post-transplant variables (rejection, immunosuppression, ABO desensitization, cumulative doses of steroid, lymphocyte depleting antibodies, tacrolimus, mycophenolate mofetil) (8·9·16·17·18·19·16·20·21·22).

In this cohort study, we evaluated the roles of different variables that may increase or decrease the risk of BKV infection and PyVAN in renal transplant recipients using univariable, multivariable and time-dependent analyses. In addition to examining many previously identified variables, we also analyzed for potential differences in risk associated with pre-transplantation dialysis modality, HD versus PD.

Materials and Methods

This study was reviewed by the institution's Research Ethics Board (REB) and data collection started after approval. We included all adult patients who received renal transplantation at London Health Sciences Centre between July 1 2012 and July 1 2014. We continued follow-up for one year following transplantation. Our main objective in this cohort study was to determine the risk factors of BKV infection in the first year after renal transplantation.

We estimated the association between the following variables and BKV infection: donor age, donor gender, donor vital status (living vs. deceased), recipient age, recipient gender, pre-transplant dialysis modality, HLA typing, HLA mismatch, cold ischemia time, cytomegalovirus serostatus at the time of transplantation, delayed graft function, rejection, ABO incompatibility, maintenance immunosuppression, and tacrolimus blood level.

Demographic information of recipients and donors was collected at the time of initial assessment. Our post-transplantation follow-up included physical examination, laboratory tests, evaluation of allograft function, updating the medication list, and finally documentation of all clinical findings, recommendations and treatment plans. Relevant medical history including underlying diseases, post-transplant events and biopsy-proven rejection episodes were also recorded. All information was documented in clinical assessment forms. Subsequently, we transferred all data to an electronic medical record system. New abnormal findings were explained to the patient and followed by a physician at the next scheduled clinical visit.

Blood samples were obtained at regular intervals. Laboratory follow-up included a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count), electrolytes, urea, creatinine, serum BK-PCR and whole blood tacrolimus trough concentration. Subsequently, we determined average tacrolimus level for each patient. Based on the central limit theorem, we calculated mean \pm SD of average tacrolimus levels for patients who developed or did not develop BKV infection. We performed this analysis in the first three months and then 4-12 months after transplantation.

Our routine screening protocol at LHSC includes BKV-PCR on plasma every 1-3 months in the first 2 years after transplantation and subsequently every year until the end of the 5th post-transplant year. Our protocol also includes BKV-PCR in patients with allograft dysfunction or patients who require allograft biopsy. Histopathologic assessment for BKV is routinely included in all renal allograft biopsies. In case of detectable viremia, we regularly monitored BKV viral load and allograft function.

Diagnosis of BKV infection

DNA extraction, using 400 μ l of plasma, was performed on the MagNA Pure Compact instrument (Roche Diagnostics, Indianapolis, IN) with the MagNA Pure Total Nucleic Acid Isolation Kit I (Roche); samples were eluted in 50 μ l. The RealStar[®] BKV PCR (Altona Diagnostics, Kit 1.2) assay was performed on a Lightcycler 2.0 instrument (Roche) with BK viral load reported in copies/ml based on the provided standards. The RealStar[®] BKV PCR Kit 1.2 is an *in vitro* diagnostic assay, based on real-time PCR, for the diagnosis of BKV infection and quantification of BKV DNA(23).

Statistical analysis

The dependent variable was BKV infection observed over the 12 months of follow-up. In univariable analyses, Chi-square and Fisher's exact tests were used to estimate the association between categorical variables, and t-tests and Mann-Whitney tests were used with continuous variables. Relative risk was calculated as ratio of the probability of BKV infection occurring in patients who were exposed to a probable risk factor to the probability of BKV infection occurring in a comparison, non-exposed group. We performed time-dependent analyses including Log Rank (Mantel-Cox), Breslow (Generalized Wilcoxon) and Tarone-Ware tests. In this analysis, the variable of time was the interval between transplantation and BKV infection. In Cox proportional hazard models, we estimated the hazard ratios of BKV infection for risk factors. In our final multivariable model, we included variables that were associated with BKV infection in univariable analysis with significance level of $p < 0.1$. In all hypothesis tests, p -values < 0.05 were considered statistically significant.

Results

This cohort comprised 175 renal transplant patients, of whom 40 (22.9%) developed BKV viremia in the first year after transplantation. Of these, 5 patients developed biopsy-proven PyVAN. One out of 8 patients who received renal/pancreas transplantation developed BKV infection (12.5%). Median interval (Inter Quartile Range_{25%-75%}) between transplantation and BKV infection was 100 (61-170) days. No patient received antiviral medications with activity against BKV such as cidofovir or brincidofovir.

No patient developed allograft failure (requiring retransplantation or returning to renal replacement therapy including hemodialysis or peritoneal dialysis). One patient passed away due to complicated sepsis after *clostridium difficile* infection. This patient developed BKV infection before *clostridium difficile* infection.

Demographic variables

Table 1 provides the characteristics of recipients with BKV infection and unaffected patients. Crude (unadjusted) estimates of association (relative risks) are also provided in table 1. P-values are based on Chi-square tests.

Table 1- Characteristics of recipients with BKV infection and unaffected patients and crude (unadjusted) estimates of association (relative risks).

Variable, n (%)	No BKV infection, n=135	BKV infection, n=40	Relative risk	p-value, CI _{95%}
Donor gender			1.1	0.5-1.6
Male	86 (76.1)	27(23.9)		
Female	47 (78.3)	13 (21.7)		
Recipient gender			2.3	1.13-4.7
Male	79 (71.2)	32 (28.8)		
Female	56 (87.5)	8 (12.5)		
Donor				0.3
DCD	47 (77)	14 (23)		
NDD	57 (73.1)	21(26.9)		
Living donor	31 (86.1)	5 (13.9)		
Living donor vs. Non-living donor	31(86.1) 105 (74.8)	5(13.9) 35 (25.2)	0.5	0.2-1.3
CMV serostatus*				0.2
D+/R+	29 (76.3)	9 (23.7)		
D+/R-	29 (69)	13 (31)		
D-/R-	42(79.2)	11 (20.8)		
D-/R+	35 (87.5)	5 (12.5)		
CMV D+/R- vs Other	29 (69) 106 (79.7)	13(31) 27 (20.3)	1.5	0.9-2.6
DGF	23 (79.3)	6 (20.7)	0.9	0.4-1.9
Blood groups, recipients				0.2
A	52 (76.5)	16 (23.5)		
B	18 (64.3)	10 (35.7)		
AB	6 (100)	0		
O	59 (80.8)	14 (19.2)		
O vs. non-O	59(80.8) 76 (74.5)	26 (25.5)	0.7	0.4-1.4
B vs. non-B	18 (64.3) 117 (79.6)	10 (35.7) 30(20.4)	1.7	0.96-3.1
Blood groups, donors				0.06
A	49 (76.6)	15(23.4)		
B	13(56.5)	10(43.5)		
AB	3(100)	0		
O	68(81.9)	15(18.1)		
B vs. Non-B	13(56.5) 120 (80)	10(43.5) 30 (20)	2.2	1.2-3.8
ABO				
Compatible	131 (76.6)	40 (23.4)	0.7	0.05-9.0
Incompatible	2 (100)	0		
Dialysis				0.06
Hemodialysis (HD)	81 (71.7)	32 (28.3)		
Peritoneal dialysis (PD)	40 (87)	6 (13)		
Preemptive (PE)	14 (87.5)	2 (12.5)		
HD vs. Non-HD [©]	81(71.7) 54(87.1)	32(28.3) 8(12.9)	2.2	1.1-4.5
Retransplantation			0.6	0.2-1.5
Yes	24 (85.7)	4 (14.3)		
No	111 (75.5)	36 (24.5)		
Allograft rejection	36 (26.7)	5 (12.2%)	0.5	0.2-1.1

*Serostatus for 2 patients were indeterminate; © Comparison HD vs. non-HD including PD and PE,

p=0.02

Regarding quantitative variables, table 2 provides the difference between recipients with BKV infection and unaffected patients. These variables include recipient age, donor age, cold ischemia time, interval between transplantation and ureteral stent removal, tacrolimus trough levels in the first 3 months and 4-12 months after transplantation. Mann-Whitney tests were used to compare patients who did and did not develop BKV infection.

Table 2- Comparison of quantitative variables between recipients with BKV infection and unaffected patients

Variable	No BKV infection, n=135	BKV infection, n=40	p-value
Median age, years (range)			
Recipients			
Overall	56 (16-82)	52 (14-79)	0.07
From deceased donors	56 (16-82)	54 (14-79)	0.3
From living donors	48 (26-71)	56 (29-67)	0.2
Median age, years (range)			
Donors			
Overall	49 (1-72)	51 (12-74)	0.4
Deceased	49 (1-69)	49 (12-74)	0.9
Living	49 (26-71)	60 (58-64)	0.0001
Median cold ischemia time (minutes)	480(14-1440)	605(168-1290)	0.4
Median interval between transplantation and ureteral stent removal, (range)	35 (12-68)	37 (22-82)	0.7
Median trough tacrolimus level (range) in the first 3 months after transplantation, ng/ml	6.05 (3.9-10.9)	6.1 (4.3-8.8)	0.4
Median trough tacrolimus level (range) 4-12 months after transplantation, ng/ml	5.6(3.1-9.0)	5.6(3.6-9.5)	0.4

Recipient HLA

Table 3 demonstrates the association between HLA mismatches and BKV infection. In this table patients with one or two mismatches were compared with no mismatch. Table 4 shows the frequencies of specific HLAs in recipients who developed BKV infection or remained BKV free.

HLA- Cw4 (RR: 2.3, CI_{95%}: 1.4-3.8) and HLA-B35 (RR: 2.5, CI_{95%}: 1.4-4.2) were significantly associated with BKV infection. However, HLA- Cw6 (RR:1.6, CI_{95%}: 0.9-2.8) and HLA-Cw7 (RR: 1.09, CI_{95%}: 0.6-1.8) did not increase the risk. P-values are based on Chi-square tests.

Table 3- Impact of HLA mismatching on the incidence of BKV infection.

VARIABLE	NO BKV INFECTION, N=135	BKV INFECTION, N=40	P-VALUE
HLA-A mismatch			0.7
0	19 (82.6)	4 (17.4)	
1	53 (75.7)	17(24.3)	
2	60 (75.9)	19 (24.1)	
HLA-B mismatch			0.6
0	10 (83.3)	2 (18.7)	
1	38 (73.1)	14 (26.9)	
2	83 (77.6)	24 (22.4)	
HLA-C mismatch *			0.03
0	20 (80)	5 (20)	
1	43 (66.2)	22 (33.8)	
2	69 (84.1)	13 (15.9)	
≤1	63(70)	27(30)	0.03
2	69(84.1)	13(15.9)	
Total class I mismatch			0.3
0	5(71.4)	2 (28.6)	
1	2(100)	0	
2	12 (92.3)	1 (7.7)	
3	14.9 (60.9)	9 (39.1)	
4	33 (76.7)	10 (23.3)	
5	38 (76)	12 (24)	
6	28 (82.4)	6 (17.6)	
<3	19 (86.4)	3 (13.6)	0.2
≥ 3	113 (75.3)	37 (24.7)	
HLA-DR mismatch			0.7
0	20 (80)	5 (20)	
1	42 (73.7)	15 (26.3)	
2	70 (77.8)	20 (22.2)	
HLA-DQ mismatch			0.2
0	26 (78.8)	7 (21.2)	
1	53 (70.7)	22 (29.3)	
2	53 (82.8)	11 (17.2)	

* Recoding of the variable to ≥ 1 mismatch did not show a significant difference between patients who developed and did not develop BKV infection (RR=0.8, CI_{95%}:0.4-1.9).

Table 4- The association between HLAs in recipients and BKV infection

VARIABLE, N (%)	NO BKV INFECTION, N=131	BKV INFECTION, N=41	RELATIVE RISK	CI _{95%}
HLA-A1			1.7	1.1-2.9
Absent	97 (81.5)	22 (18.5)		
Present	38 (67.9)	18 (32.1)		
HLA-A2			0.9	0.6 -1.8
Absent	74 (77.1)	22 (22.9)		
Present	61 (77.2)	18 (22.8)		
HLA-A3			0.9	0.5-1.9
Absent	107 (77)	32 (23)		
Present	28 (77.8)	8 (22.2)		
HLA-B8			1.2	0.7-2.3
Absent	106 (77.9)	30 (22.1)		
Present	26 (72.2)	10 (27.8)		
HLA-B35			2.5	1.4-4.2
Absent	120 (80.5)	29 (19.5)		
Present	12 (52.2)	11 (47.8)		
HLA-Cw4			2.3	1.4-3.8
Absent	113 (81.3)	26 (18.7)		
Present	19 (57.6)	14 (42.4)		
HLA-Cw6			1.6	0.9-2.8
Absent	110 (79.1)	29 (20.9)		
Present	22 (66.7)	11 (33.3)		
HLA-Cw4 or Cw6			2.7	1.6-4.7
Absent	95 (85.6)	16 (14.4)		
Present	37 (60.7)	24 (39.3)		

Table 5- Correlations between variables

	Recipient age	HLAC4	HLAB35	HD vs NON-HD	Gender	Donor BG B vs non-B	HD_HLA C4	HLA B35_C4	HLAA1	HLA C two MM or NOT
Correlation	1	.017	-.028	.034	.012	-.052	.029	.003	.008	.170 [*]
Recipient age Sig. (2-tailed)		.823	.711	.654	.873	.500	.706	.971	.918	.026
N	175	172	172	175	175	173	172	172	175	172
Correlation	.017	1	.749 ^{**}	-.124	.099	.103	.934 ^{**}	.772 ^{**}	-.127	.023
HLAC4 Sig. (2-tailed)	.823		.000	.106	.196	.181	.000	.000	.097	.763
N	172	172	172	172	172	170	172	172	172	172
Correlation	-.028	.749 ^{**}	1	-.077	.117	.045	.726 ^{**}	.975 ^{**}	-.091	.104
HLAB35 Sig. (2-tailed)	.711	.000		.315	.126	.563	.000	.000	.237	.175
N	172	172	172	172	172	170	172	172	172	172
Correlation	.034	-.124	-.077	1	-.008	-.035	.025	-.066	.055	-.075
HD vs NON-HD Sig. (2-tailed)	.654	.106	.315		.915	.648	.740	.393	.467	.328
N	175	172	172	175	175	173	172	172	175	172
Correlation	.012	.099	.117	-.008	1	.088	.095	.106	.063	.086
Gender Sig. (2-tailed)	.873	.196	.126	.915		.247	.214	.165	.407	.261
N	175	172	172	175	175	173	172	172	175	172
Correlation	-.052	.103	.045	-.035	.088	1	.043	.052	-.016	.031
Donor BG B vs non-B Sig. (2-tailed)	.500	.181	.563	.648	.247		.579	.497	.833	.686
N	173	170	170	173	173	173	170	170	173	170
Correlation	.029	.934 ^{**}	.726 ^{**}	.025	.095	.043	1	.748 ^{**}	-.110	-.001
HD-C4 Sig. (2-tailed)	.706	.000	.000	.740	.214	.579		.000	.151	.995
N	172	172	172	172	172	170	172	172	172	172
Correlation	.003	.772 ^{**}	.975 ^{**}	-.066	.106	.052	.748 ^{**}	1	-.080	.088
HLA B35_C4 Sig. (2-tailed)	.971	.000	.000	.393	.165	.497	.000		.295	.254
N	172	172	172	172	172	170	172	172	172	172
Correlation	.008	-.127	-.091	.055	.063	-.016	-.110	-.080	1	-.042
HLAA1 Sig. (2-tailed)	.918	.097	.237	.467	.407	.833	.151	.295		.583
N	175	172	172	175	175	173	172	172	175	172
Correlation	.170 [*]	.023	.104	-.075	.086	.031	-.001	.088	-.042	1
HLA C two MM or NOT Sig. (2-tailed)	.026	.763	.175	.328	.261	.686	.995	.254	.583	
N	172	172	172	172	172	170	172	172	172	172

*. Correlation is significant at the 0.05 level (2-tailed).

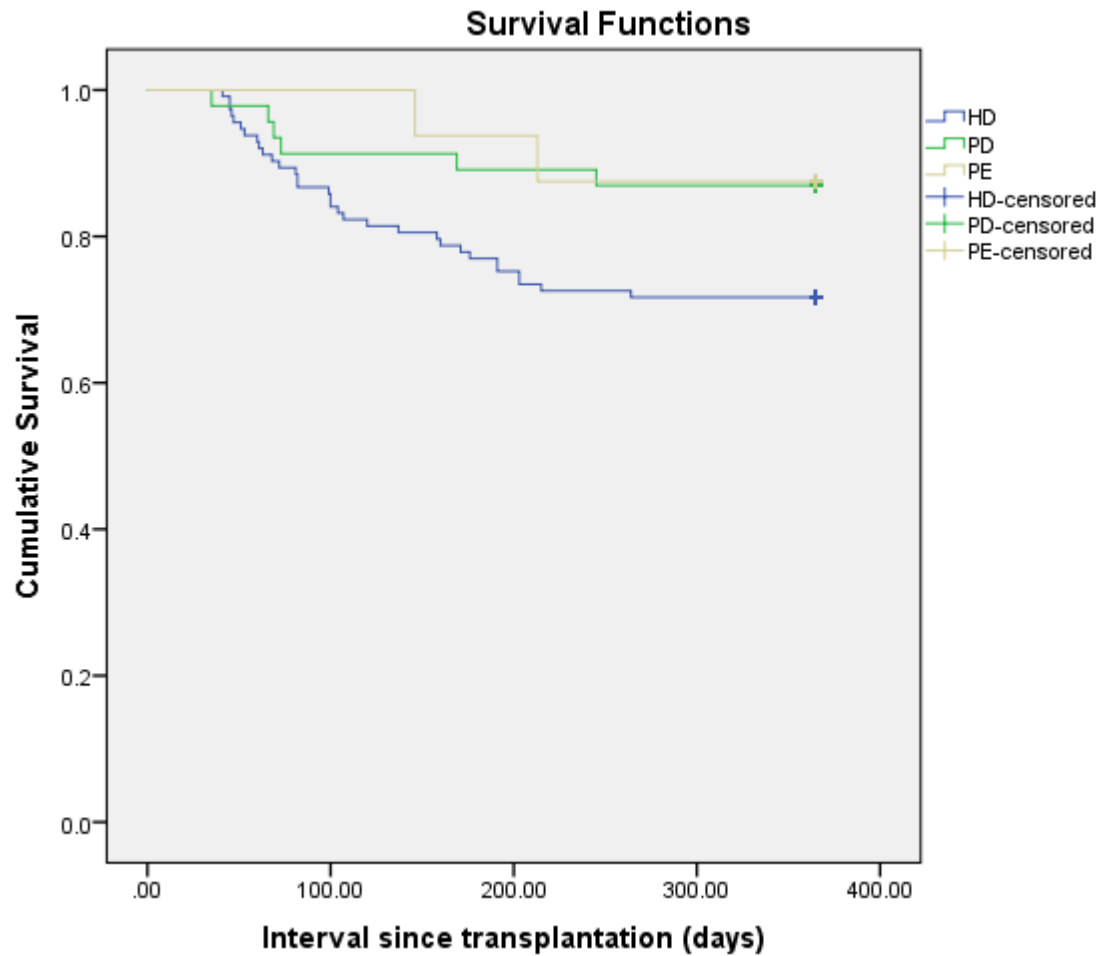
**. Correlation is significant at the 0.01 level (2-tailed).

Survival analysis

We analyzed the effect of hemodialysis (HD) on the time- to-event of BKV infection. We also compared this effect with the effects of preemptive transplantation (PE) and peritoneal dialysis (PD). Figure 1 demonstrates the survival functions of HD, PD and PE on the time-to-event of interest (BKV infection).

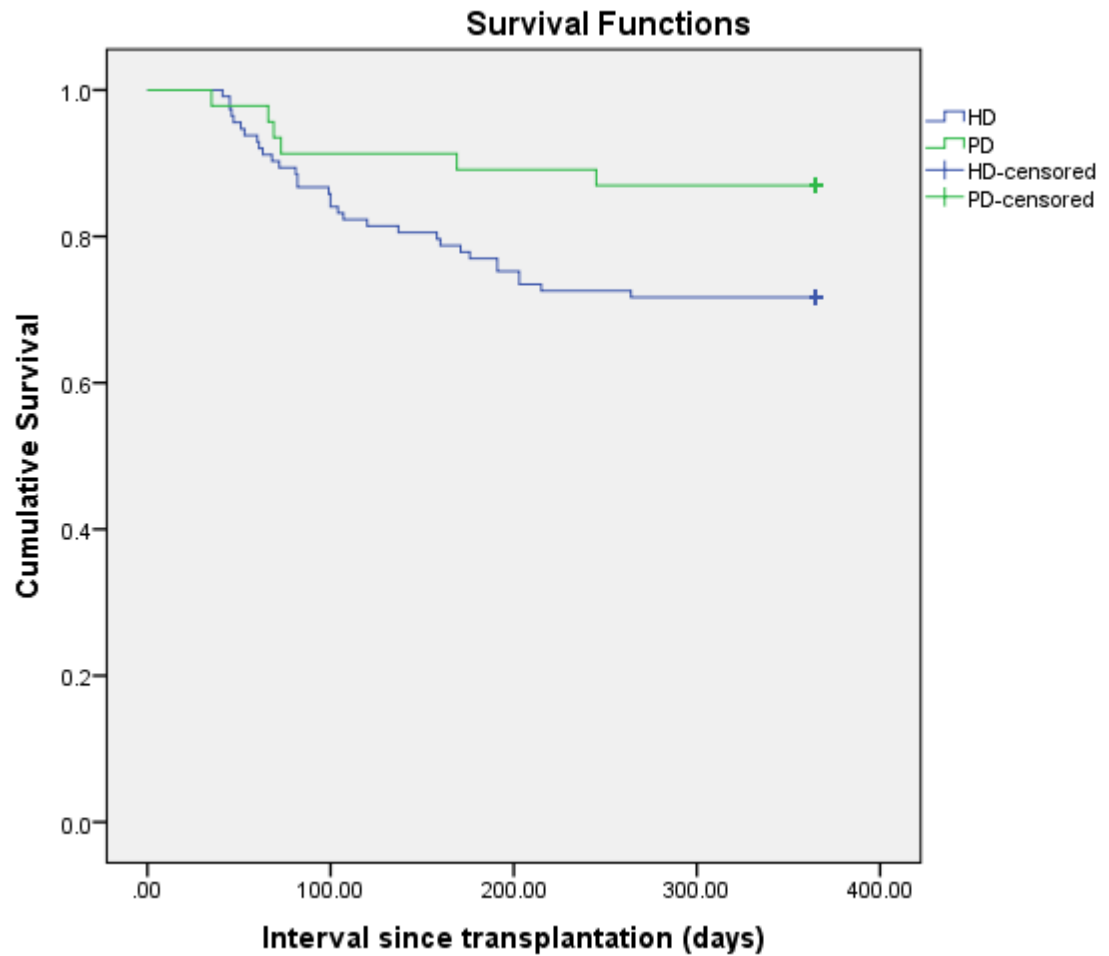
We repeated the analysis comparing the patients who received HD and PD before transplantation. Figure 2 demonstrates the survival functions of HD vs. PD on the time-to-event of interest (BKV infection). Log Rank (Mantel-Cox), Breslow (Generalized Wilcoxon) and Tarone-Ware tests all showed a significant difference between HD and PD patients. There was no significant difference between PE and HD patients with a higher risk associated with pre-transplantation HD. Figure 3 demonstrates this finding in detail.

We performed similar analysis to compare the effect of living donors (LD) vs. non-LD (DCD and NDD). Figure 4 shows receiving organ from LD vs. non-LD did not significantly change the risk of BKV infection in time-dependent analysis.



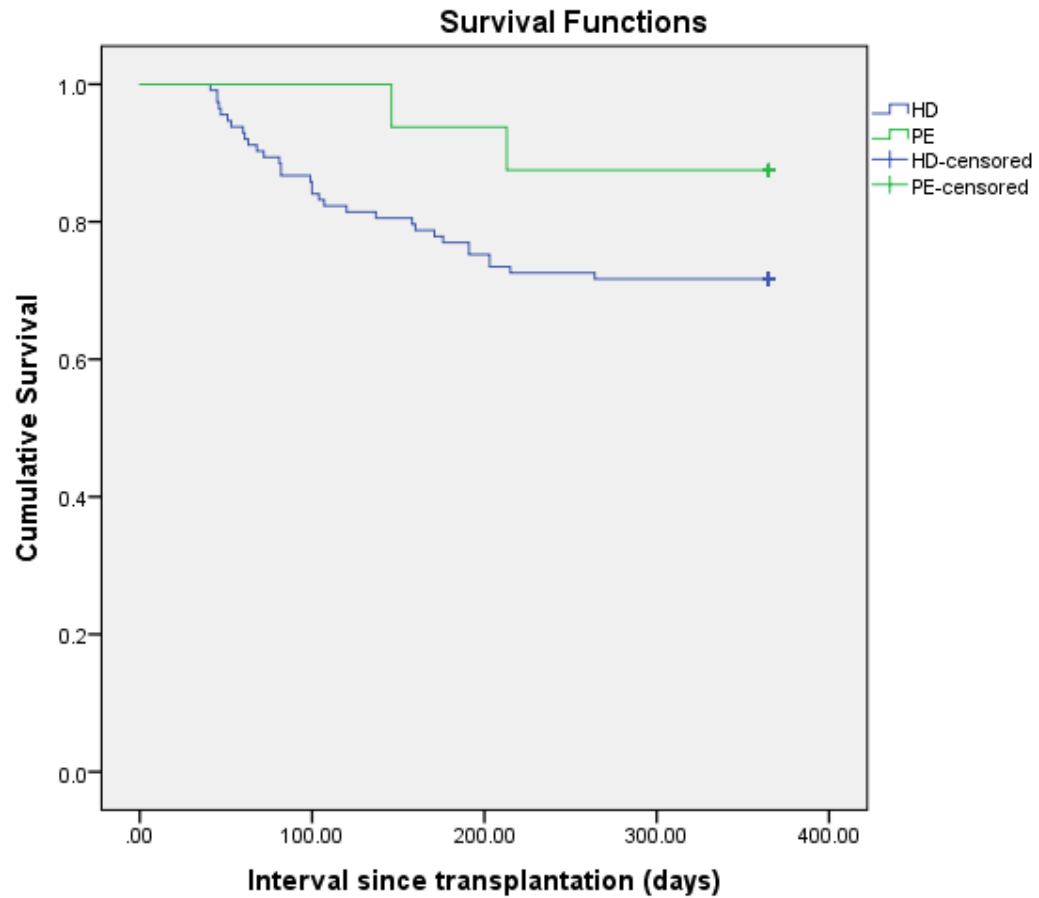
	Chi-Square	Degree of freedom	p-value
Log Rank (Mantel-Cox)	5.32	2	.07
Breslow (Generalized Wilcoxon)	5.38	2	.07
Tarone-Ware	5.36	2	.07

Figure 1: Survival functions in those receiving preemptive transplantation (PE) and two methods of renal replacement therapies before transplantation including hemodialysis (HD) and peritoneal dialysis (PD) on post-transplant BKV infection.



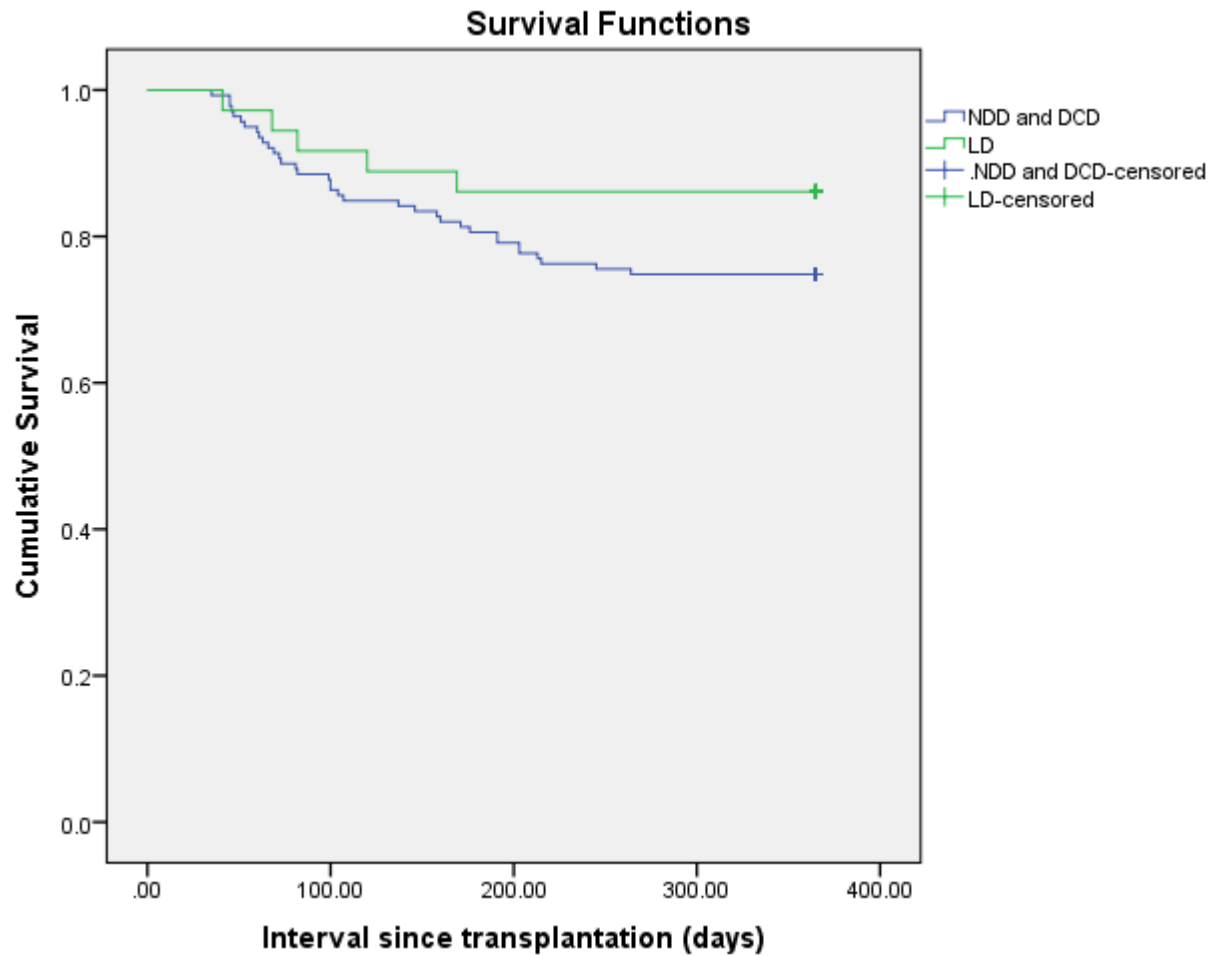
Test	Chi-Square	Degree of freedom	p-value
Log Rank (Mantel-Cox)	3.93	1	.05
Breslow (Generalized Wilcoxon)	3.79	1	.05
Tarone-Ware	3.87	1	.05

Figure 2: Survival functions in those receiving pretransplant peritoneal dialysis (PD) vs. hemodialysis (HD) on post-transplant BKV infection



Test	Chi-square	Degree of freedom	p value
Log Rank (Mantel-Cox)	1.84	1	.17
Breslow (Generalized Wilcoxon)	2.06	1	.15
Tarone-Ware	1.96	1	.16

Figure 3: Survival functions in those receiving preemptive transplantation (PE) vs. pretransplant hemodialysis (HD) on post-transplant BKV infection



Test	Chi-square	Degree of freedom	p-value
Log Rank (Mantel-Cox)	1.88	1	.17
Breslow (Generalized Wilcoxon)	1.77	1	.18
Tarone-Ware	1.83	1	.18

Figure 4: Survival functions in recipients of living donor (LD) vs. Donation after cardiac death (DCD) and Donation after neurological determination of death (NDD)

Multivariable analysis

We performed Cox proportional hazard test in multivariable analysis. In univariable analyses, we found significant roles of HLA- B35, HLA-Cw4, HLA-C mismatch, chronic pretransplant hemodialysis, donor blood group B, and recipient gender in post-transplant BKV infection. Two mismatches of HLA-C vs. no mismatch provided a protective effect in univariable analysis. We forced recipient age ($p=0.08$ in univariable analysis) in all multivariable models.

Since patients have combinations of different HLA molecules, interaction between HLA variables was expected. We initially calculated Pearson correlation coefficients to examine interactions between variables. The result of these tests showed a significant interaction between HLA B35 and Cw4 ($p=0.0001$). Both variables had significant associations with BKV infection in univariable analysis. Considering the interaction between these two variables, we made a new variable of “B35/Cw4” (0=B35-/Cw4-, 1=B35+/Cw4- or B35-/Cw4+ and 2=B35+/Cw4+). In univariable analysis, B35/Cw4 +/+ significantly increased the risk of BKV infection (HR: 3.2, CI_{95%}: 1.6-6.5).

We did not include “donor blood group B vs. non-B” in the model despite significant association with BKV infection in univariable analysis. This was because the non-B group included heterogeneous patients with different risks of rejection and graft loss (blood group A, AB and O).

Cox proportional hazard model

Table 6 provides our findings in Cox proportional hazard regression analysis in detail. Male gender (HR: 2.95, CI_{95%}: 1.3-6.6), pre-transplant HD vs. non-HD (HR: 3.04, CI_{95%}: 1.4-6.8), HLA-A1 (HR: 3.06, CI_{95%}: 1.5-6.2), HLA B35/Cw4 +/+ (HR: 4.6, CI_{95%}: 2.1-10.1) significantly increased the risk of BKV infection. In this model, there was a quantitative association between recipient age and BKV infection (HR: 1.04, CI_{95%}: 1.01-1.06). Two HLA mismatches of HLA-C provided a protective effect in this time-dependent analysis (HR_{one mismatch}: 1.2, CI_{95%}: 0.4-3.3, HR_{two mismatch}: 0.3, CI_{95%}: 0.1-0.98).

Table 6: Cox proportional hazard analysis

Variables	Sig	HR	95.0% CI for HR	
			Lower	Upper
Gender male	.008	2.95	1.32	6.57
Pre-transplant chronic hemodialysis	.006	3.04	1.37	6.77
HLAB35/Cw4 ^Ω	.001			
HLAB35/Cw4 (+/- or -/+)	.758	1.22	.35	4.22
HLAB35/Cw4 (+/+)	.000	4.63	2.12	10.14
HLA A1	.002	3.06	1.51	6.17
HLA C mismatch	.001			
HLA C one mismatch	.749	1.18	.42	3.31
HLA C Two mismatch	.046	.32	.107	.98
Recipient age (per year)	.006	1.04	1.01	1.06

Ω Having either HLA B35 or HLA Cw4:

-/-: the recipient had none of these two HLAs (reference in the analysis)

+/- or -/+: the recipient had either one of these two HLAs

+/+ : the recipient had both HLAs

Discussion

In this cohort, 175 renal allograft recipients were included. We continued one-year of follow-up after transplantation. The frequency of BKV infection in the first year after transplantation affects almost one quarter of patients (23%). BKV infection in our cohort was more frequent than previous reports (24,25,26) but was similar to a recent report by Hirsch et al(27). Serial post-transplantation screening showed 50% of BKV infections occurred in the first 100 days and 75% in the first 170 days after transplantation (IQR 25%-75%: 62-170 days). Based on Organ Procurement and Transplantation Network (OPTN) database with two- year follow up, 3.45% of renal transplant recipients in the United States required treatment for BKV infection. This frequency increased to 6.6% at 60 months(22). OPTN data was limited to patients who required treatment for BKV infection and not necessarily diagnosis of BK viremia. Considering the frequency of BK viremia in our data, it appears post-transplant BKV infection is a considerable problem in London, Ontario affecting approximately one fourth of renal transplant recipients. The reason for this high frequency of BKV infection is unclear to the authors. This frequency of BK infection could be due to using highly sensitive diagnostic tests and a rigorous screening protocol. However, the molecular PCR test that we use in London is similar to other transplant centers in Canada. Further studies are needed to determine the reason(s) for such a frequency of BKV infection at London Health Science Centre.

Although a recent study suggested BK viremia could occur independent of recipient characteristics(28), we found a significant association between recipient age and post-transplant BKV infection in Cox proportional hazard model. Since cell mediated immunity is affected by age, this finding does not appear to be unexpected.

Additionally, in patients who received an organ from living donors, the median donor age was significantly higher in recipients with BKV infection. It has been shown that BK virus infection preferentially affects older recipients (29). It was also shown the frequency of BK viraemia gradually increases with age even in immunocompetent patients(30). As well, the frequency of BKV infection in pediatric renal transplant recipients appears to be considerably lower than adult patients(31). All these data support the association between age and BKV infection. We hope further studies can provide risk stratification based on age of the donors and recipients.

In this study, cold ischemia time and allograft rejection were not significantly associated with BKV infection. This finding supports similar data published by Priftakis et al(32). On the other hand, Dharnidharka et al(22) showed acute rejection in the first 6 months after transplantation may increase the risk of BKV infection. However, the authors identified a high frequency of HLA mismatch in that study and they believed the effect of rejection could be due to considerable frequency of HLA mismatch in patients with BKV infection. Although reducing immunosuppression after the diagnosis of BKV infection increases the risk of rejection, there is no clear data to show a reverse association suggesting allograft rejection is a risk factor for BKV infection or PyVAN.

It has been shown that receiving tacrolimus increases the risk of BKV infection compared by cyclosporine(25). All patients in this cohort received tacrolimus and hence we were not able to compare the effect of tacrolimus with other CNIs. We were interested in demonstrating a dose dependent effect of tacrolimus on BKV infection. However, our data showed no quantitative association between tacrolimus serum level and BKV infection.

We were not able to determine area under curve (AUC) and maximal concentration (C_{max}) of the medication to show the magnitude of exposure. We believe further pharmacokinetic studies are required to investigate the quantitative association between immunosuppression and BKV infection.

In our study, HLA-A1 was significantly associated with post-transplant BKV infection. HLA-A1 has been recently shown to be associated with other post-transplant viral infections such as EBV related lymphoma (33), HSV(34), and CMV (35) infection. In addition, we found B35 and Cw4 increase the risk of BKV infection following renal transplantation. Due to an interaction between B35 and Cw4 in statistical analysis, we made a combined variable of B35/Cw4. Interestingly, we found a significant risk of BKV infection in recipients who have both HLA B35 and Cw4 (B35/Cw4 +/+). The role of HLA-B35 has been recently demonstrated in other viral diseases and specifically HIV infection(36,37). It appears HLA-B35 increases the susceptibility to BKV infection as well. This HLA seems to be frequent in the Canadian population(38). On the other hand, HLA-Cw4 has been previously shown to be associated with autoimmune diseases(38,39). The association of HLA A1, B35 and Cw4 remained significant in the Cox proportional hazard model. Further studies are required to determine the roles of these HLA variables in BKV infection.

A higher frequency of HLA mismatch has been identified as a risk factor for BKV infection in either retrospective or prospective studies(40,18). In our study, HLA mismatch was not associated with post-transplant BKV infection. Our finding is similar to some other studies that did not show HLA mismatching as a risk factor for BKV infection(41,42,11). The protective effect of mismatching of two HLA-C which was found in Cox proportional hazard model does not seem biologically plausible.

Our study showed the frequency of BKV infection in patients who received rATG was not significantly different from patients who did not receive this medication. This finding was similar to OPTN data which showed a non-significant effect of induction with rATG(44). The maintenance immunosuppressive protocol was similar for all renal allograft recipients during this cohort. Thus, we were not able to compare the effects of immunosuppressive regimens. This is one of the limitations of this study.

To our knowledge, this is the first study that compared the baseline risk of BKV infection between hemodialysis (HD) and peritoneal dialysis (PD) patients. Mitterhofer et al showed a low frequency of BKV infection in PD patients in a descriptive study(45). In our study, the risk of BKV infection in HD patients was significantly higher than PD patients in univariable analysis. In survival analysis, we showed different survival function of the outcome of BKV infection considering pretransplant PD vs. HD. Finally, the Cox proportional hazard model showed the risk of BKV infection is significantly higher in HD patients. To our knowledge, this is the first prospective study that showed significantly different baseline risk of BKV infection between HD and PD patients. Other investigators have shown a difference between HD and PD patients in terms of the function of the cell mediated immune system(46). This discrepancy may explain why HD patients are at a higher risk of BKV infection compared to PD patients. The process of hemodialysis seems to be associated with chronic activation of the immune system causing a chronic inflammatory response. The causes and consequences of hypercytokinemia in HD patients remains unknown(47). Further studies with a specific focus on chronic inflammatory response and function of cell mediated immunity in uremic patients may explain their vulnerability to BKV infection.

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CHAPTER 4: GENERAL DISCUSSION AND CONCLUSION

In this cohort study, we evaluated the roles of different variables that may increase the risk of BKV infection and PyVAN in renal transplant recipients using univariable, multivariable and time-dependent analyses.

Our objective was to determine the risk factors for BKV infection (presumptive polyomavirus associated nephropathy, PyVAN) in renal allograft recipients. We believe the age of the recipient and recipient gender are two important variables that should be considered in risk stratification and screening strategies. The effects of these two variables have been demonstrated in previous studies(1,2). It was also assumed a sexually transmitted trigger for reactivation of BK disease (3, 4). Our data may support such a hypothesis.

In addition to examining many variables that were previously identified, we also analyzed potential differences in risk associated with pre-transplantation dialysis modality, HD versus PD. PD and HD patients do not appear to have an equal baseline risk for BK virus infection at the time of transplantation. Chronic renal failure is accompanied by different immunologic abnormalities of innate and acquired immunity(5). These abnormalities include lymphopenia, altered percentage values of CD3+, CD3+/ 4+, CD19+ subpopulations and increased percentages of natural killer cells(6). Soluble IL-2 receptors are usually elevated in patients with ESRD. However, this level is considerably higher in PD patients rather than HD(7). HD patients have a significantly lower percentage of CD3-positive cells than PD patients(7). This baseline characteristic of HD patients may cause more replication of BKV in their urothelial cells before transplantation.

The process of apoptosis also seems less affected by PD than HD. It has been shown

that FAS expression is considerably higher in PD than in HD patients(8). The pattern of cytokine production by CD4-positive cells shows a significantly higher percentage of a Th1-type cytokine production in HD patients and Th2-type cytokine secretion in PD patients(9). This dissimilarity of immune system function between HD and PD patients may explain their different baseline risks of BKV infection at the time of transplantation. We believe further studies should focus on dysfunction of the immune system in uremic patients to explain the protective effect of PD vs. HD.

We believe our finding related to the association between HLA-B35/ Cw4 and BK virus infection may suggest the role of killer cell immunoglobulin-like killer receptors (KIR). Such an association has been shown in other infectious diseases such as tuberculosis(10,11,12,13). KIR is responsible for checking the existence of MHC class I molecules, which function as their ligands. Although KIR receptors were previously recognized as specific for NK cells, currently these receptors are known to also present on T cells(14). Genetics of KIR molecules is very complex and that creates great variability. Additionally, some KIR are known to recognize HLA-A, HLA-B or HLA-C molecules(15). This makes a huge diversity of reactions, depending on the presence or absence of given KIR and their ligands, and therefore different susceptibility to BK virus infection. The interaction that we found in this study between HLA-B35 and HLA-C4 strongly suggests a probable role of KIR. We believe further studies are required to investigate the role of KIR in post-transplant BKV infection.

There are a number of potential limitations that could be associated with our study as an observational research. These limitations did not necessarily exist in this project but should be considered as potential issues.

An important one relates to my role as the observer while I was involved in the process of diagnosis and treatment of BKV infection. It was not possible to blind (i.e., keep the exposure status of the study participant unknown) the research team who were assessing the study outcome. Although we did our best to follow the protocol, knowing the exposure status of the study participant (PD vs HD) might have influenced the assessment of the outcome.

Secondly, although we followed LHSC protocol for diagnosis of BKV infection in renal transplant recipients, it is possible that some patients more frequently performed the diagnosis tests. On the other hand, it is also possible that the diagnosis of BKV infection was delayed in some patients. This is specifically true for some patients who did not attend in the clinic in regular intervals.

As an observational study, the existence of alternative explanations for study results due to confounding was carefully considered. To our knowledge, other than transplantation, no alternative explanation for BKV infection has been introduced in renal allograft recipients. The other limitation of observational studies is loss to follow-up. No patient in this study discontinued follow-up in the first year after transplantation. According to our protocol, recipients who were supposed to continue post-transplant follow-up in other medical centers were excluded.

It is important to consider that our study was an explanatory research. Further studies are required to determine the roles of independent variables such as HD vs PD to predict the outcome of BKV infection in renal transplant recipients.

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APPENDICES

Appendix A: Summary of study methodology

1. Type of study

Single group cohort

2. Data collection method

We routinely collected the demographic data of the donors and the recipients. Dr Q Xu kindly provided the HLA data. These laboratory data were related to both donors and recipients in detail. As a result, we regularly collected all background information related to the renal allograft recipients and the donors.

Inpatient Transplant Infectious Diseases consultation was routinely done after renal transplantation. We subsequently arranged follow-up visits at Transplant Infectious Diseases and Nephrology clinics at LHSC. In each visit, we reviewed symptoms, performed physical examination and monitored allograft function. We regularly performed laboratory tests to monitor serum electrolytes and tacrolimus levels.

Our routine BKV screening protocol at LHSC includes BKV-PCR on plasma every 1-3 months in the first 2 years after transplantation and subsequently every year until the end of the 5th post-transplant year. Our protocol also includes BKV-PCR in patients with allograft dysfunction or patients who require allograft biopsy. Histopathologic assessment for BKV is routinely included in all renal allograft biopsies. In case of detectable viremia, we regularly monitored BKV viral load and allograft function.

We implemented regular screening strategy for all renal allograft recipients in June 2012. As a routine, I received a copy of all plasma BK virus PCR results during this cohort.

In case of a positive result, we informed the patient and continued active follow-up. All data were transferred to an Excel spreadsheet.

3. Inclusion Criteria

1. Age \geq 18 years
2. Renal or renal/pancreas transplantation from living or deceased donors
3. Receiving renal allograft at London Health Sciences Center (LHSC)
4. Patient agrees to continue follow up at LHSC

4. Exclusion Criteria

1. Pediatric renal transplant patients
2. Hematopoietic stem cell transplant patients
3. Patients who receive renal allograft in other transplantation centers and continued post-transplantation follow-up at LHSC
4. Patients who received allografts other than kidney or kidney-pancreas transplantation

5. Polyomavirus (BK virus) PCR

DNA extraction, using 400 μ l of plasma, was performed on the MagNA Pure Compact instrument (Roche Diagnostics, Indianapolis, IN) with the MagNA Pure Total Nucleic Acid Isolation Kit I (Roche); samples were eluted in 50 μ l. The RealStar[®] BKV PCR (Altona Diagnostics, Kit 1.2) assay was performed on a Lightcycler 2.0 instrument (Roche) with BK viral load reported in copies/ml based on the provided standards. The RealStar[®]BKV PCR

Kit 1.2 is an *in vitro* diagnostic assay, based on real-time PCR, for the diagnosis of BKV infection and quantification of BKV DNA

6. Statistical Software

We used IBM SPSS Statistics version 24 (2015) for data analysis.

7. Data analysis

- I. Categorical variables: Absolute and relative frequencies, cumulative percent
- II. Quantitative variables: Mean, median, standard deviation, variance, mode, skewness, range, IQR
- III. Analytic analysis: chi-square test, Fisher's exact test, cox proportional hazard regression, logistic regression, Mann-Whitney test

8. Multivariate analysis

We provided the results of Cox proportional hazard analysis which is a time-dependent test in Chapter 3. The following table demonstrates our findings of multivariate logistic regression test in detail. All variables that had associations with BK virus infection in univariate analyses ($p < 0.1$) were included in this model. Despite Cox regression model, the logistic regression analysis is not time-dependent.

9. Sample size, type I and type II errors:

The desired power was 0.8 (type II error, $\beta = 0.2$). Considering the confidence level of 0.95 and the expected incidence of 0.15 in this cohort, the calculated sample size was 114.

Table 7- Logistic regression analysis

Variable	Sig.	Odds ratio	95% C.I. for OR	
			Lower	Upper
Gender	.012	3.57	1.32	9.68
HLAB35/Cw4	.004			
HLAB35/Cw4 (+/-, -/+)	.870	1.14	.24	5.44
HLAB35/Cw4(+/+)	.001	6.29	2.09	18.98
HLA-A1	.012	3.10	1.28	7.50
HD vs Non-HD	.010	3.59	1.35	9.51
Locus Cw MM ^{CE}	.007			
Locus Cw MM(1)	.681	1.30	.37	4.56
Locus Cw MM(2)	.077	.30	.08	1.14
Recipient age	.014	1.04	1.01	1.07
Constant	.0001	.004		

Ω Having either HLA B35 or HLA Cw4:

-/-: the recipient had none of these two HLAs (reference in the analysis)

+/- or -/+: the recipient had either one of these two HLAs

+/+ : the recipient had both HLAs

CE MM: mismatch

MM(1): one mismatch

MM(2): two mismatches

Appendix B

REB approval

ROMEO - Researcher Portal General Info

File No: 105559 Title: BK virus infection in the first year after renal transplantation in patients who were on hemodialysis vs. peritoneal dialysis before transplantation Start Date: 19/08/2014 End Date: 19/08/2017

Keywords: BK virus, renal, transplantation, hemodialysis, peritoneal dialysis



**Western
Research**

Research Ethics

**Western University Health Science Research Ethics Board
HSREB Delegated Initial Approval Notice**

Principal Investigator: Dr. Seyed Hosseini-Moghaddam

Department & Institution: Schulich School of Medicine and Dentistry/Medicine-Dept of London Health Sciences Centre

HSREB File Number: 105559

Study Title: BK virus infection in the first year after renal transplantation in patients who were on hemodialysis vs peritoneal dialysis before transplantation

Sponsor:

HSREB Initial Approval Date: August 19, 2014

HSREB Expiry Date: June 30, 2015

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Other	Data Collection Form	2014/07/21
Western University Protocol		2014/08/11

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review. If an Updated Approval Notice is required prior to the HSREB Expiry Date, the Principal Investigator is responsible for completing and submitting an HSREB Updated Approval Form in a timely fashion.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH GCP), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

_____, on behalf of Dr. Joseph Gilbert, HSREB Chair

Ethics Officer to Contact for Further Information

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