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Risk factors for BK viremia in kidney transplant recipients

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Abstract

Background. In the past 20 years, BK virus has emerged as a cause of early graft dysfunction after kidney transplantation. In the setting of chronic immunosuppression (IS), the latent virus can reactivate, leading to BK viremia (10-20%) and in 1-10% of kidney transplant recipients to BK virus nephropathy (BKVN). The early detection of BK viremia by serum DNA PCR screening allows prompt but controlled reduction of IS, which, despite numerous attempts to find specific antiviral agents, remains the mainstay therapy. So far, besides potent IS, no risk factor has been consistently associated with BK viremia/BKVN. The use of a ureteral stent at the time of transplantation to protect the ureterovesical anastomosis has been described as a potential trigger. In this study, we aimed at defining the incidence and kinetics of BK viremia in our local cohort of kidney transplant recipients, and analysed potential predictors of BK viremia/BKVN, including ureteral stents.

Methods. We performed a single-centre retrospective study on consecutive patients who received a kidney transplant at the CHUV between 01.11.03 and 31.12.12, with at least 12 months follow-up. First, descriptive statistics were done to define the general characteristics of the population. From a total 308 patients, a subpopulation of 195, transplanted between 01.01.08 and 31.12.12, had enough data for relevant analysis of BK viremia status during the first year as well as the use of a ureteral stent. Statistical analyses were performed using R-software.

Results. BK viremia (>1000 copies/ml at least twice) was detected in 37/195 (19%) patients within the first year post-transplantation, with an early onset in the first 4 months for 65%, whereas only 6 patients were newly diagnosed after 12 months. 28/195 (14.4%) had a peak BK viremia >10'000 copies/ml, which represents a high positive predictive value for BKVN. Patients with BK viremia had a significantly lower kidney function at one year as compared to BK viremia negative recipients (eGFR=58 vs. 67 ml/min; p=0.019), and eGFR decreased as viremia levels increased, in particular >10'000 copies/ml. We found no significant association with the type of graft (living vs. cadaveric donor), or IS protocols (Basiliximab vs. Thymoglobulin induction, tacrolimus vs. cyclosporine). Interestingly, combining recipient's age and gender, we observed a higher risk to reactivate BK virus in older men (p=0.05). Ureteral stents were placed in 76/195 patients (39%), but their use did not significantly influence BK viremia.

Conclusion. Considering the incidence of BK viremia in our population (22%), the fact that BKVN represents a poor prognosis factor for graft function and that viremia detection by PCR allows early diagnosis and management, our data reinforce the importance of regular screening early after kidney transplantation and in the case of unexplained rise in serum creatinine. Based on current knowledge and on our data, a prospective randomized multicentre study with controlled variables (IS, ureteral stents) and standardized follow-up charts (including urological complications/manipulations) would help better understand the determinants of BK viremia/BKVN.

Key words. Kidney transplantation, BK virus, Ureteral stent, Immunosuppression

Introduction

1. Kidney transplantation

At the end of June 2015, according to SwissTransplant statistics, 1050 patients were waiting for a kidney and registered on the waiting list. During the 2 first semesters of 2015, 162 kidney transplantations have been performed in Switzerland, 115 thanks to deceased donors and 47 from living donors, sometimes preemptively (before starting dialysis). Considering the disproportion between the patients in need of a kidney and the transplantations possibilities, it is our duty to prevent any complication that could reduce kidney graft function in the perspective of helping the transplanted patient having a longer and healthier life.

In the world of organ transplantation, the kidney plays a special role. Because of its relatively easy surgical removal and implantation (in comparison with other organs), as well as the possibility to use kidneys from living donors, it is the first organ transplanted successfully (1954). Before the discovery of the human leucocyte antigen (HLA) system (1958), a better understanding of the mechanisms of organ rejection as well as the availability of efficacious immunosuppressive protocols (Azathioprine-Prednisone in the 70's and Cyclosporine A in the 80's), successful kidney transplantations were only possible between identical twins. Progressively, kidney transplantations between HLA-mismatched donor-recipient pairs and transplantations from cadaveric donors have been successfully performed.

Nowadays, because of the many diseases that cause end stage renal disease (ESRD) and despite the access to chronic dialysis, the kidney is the more needed organ. The fact that two kidneys are available from a cadaveric donor and that living donations are possible helps to maintain the balance between supply and demand in acceptable ranges. General consensus establishes that kidney transplantation is the optimal treatment for most patients with ESRD, despite the risks associated with the transplantation surgery and lifelong immunosuppressive therapy. This procedure restores a near normal kidney function and contributes to the patient's quality of life.

The other existing renal replacement therapies are hemodialysis and peritoneal dialysis. Constant improvement in the techniques and the possibilities of at-home dialysis render dialysis a long-lasting and life-saving therapy for ESRD patients. However, because of dialysis possible side effects and non-optimal blood purification, the life expectancy of patients on chronic dialysis is significantly shorter as compared to kidney-transplanted patients, mainly due to cardiovascular complications.

Preemptive transplantation is defined as elective transplantation prior to the start of chronic dialysis and, given the long waiting lists, this procedure is generally possible by living-donor transplantation. Current data show that patients and grafts survival is longer after preemptive kidney transplantation as compared to transplantation after a period of chronic dialysis. Moreover, as the adverse effects of dialysis on post-transplant outcomes are duration dependent, transplantation should ideally be performed as soon as possible.

The type of kidney donation also influences patient and graft survival. Transplants obtained from deceased donors often suffer from prolonged ischemia time and some degree of renal impairment may also result from donor's comorbidities, older age and organ retrieval procedures (for example donation after cardiac death). In comparison, living donation allows the selection of a healthy donor for the transplantation procedure as well as planning of the surgery, allowing graft removal and transplantation under favorable conditions and shorter cold ischemic time. Thanks to minimal invasive surgical techniques and extensive preoperative examination, the removal of a kidney from a living, healthy and motivated human is acceptable. According to the United Network for Organ Sharing (UNOS), in 2001, kidney graft survival at 5 years after transplantation was 10% greater after living versus cadaveric donation (91% vs. 81%, respectively).

2. Immunosuppressive therapy after solid organ transplantation

Immunosuppression (IS) is an essential element to ensure optimal patient and graft outcome after solid organ transplantation (SOT), as it allows preventing and treating rejection episodes.

The induction therapy is given at the time of transplantation and its purpose is to improve the efficacy of immunosuppression and reduce early acute rejection episodes. Indeed, the use of potent induction therapies allows delayed introduction as well as lower doses of calcineurin inhibitors (CNI), which are potentially nephrotoxic. The induction therapy aims at depleting or modulating recipient's T-cell responses during donor's antigen presentation. Basiliximab, an anti-IL-2 receptor alpha-chain monoclonal antibody, binds this molecule, which is upregulated at the surface of activated T lymphocytes and competitively inhibits IL-2-mediated effector functions. This modulation of T cells is reversible and Basiliximab is used in patients at low immunological risk of rejection (first transplantation, non-sensitized recipients). In high immunological risk patients (second transplantation, sensitized recipients) or in the case of delayed graft function (DGF), Thymoglobulin (rabbit polyclonal anti-thymocyte globulin), a T-cell depleting agent is preferentially used. In hypersensitized recipients with pre-existing donor-specific antibodies (DSA), intravenous immunoglobulins (Ivlg) can be administered together with Thymoglobulin and/or plasma exchanges can be performed to remove DSA.

The maintenance therapy is the long-term treatment, which is necessary to down-regulate the immune system against the allograft in order to prevent acute and chronic rejection that lead to progressive graft dysfunction. It consists of a combination of 2 or 3 drugs, to achieve sufficient immunosuppression while minimizing the toxicity and adverse effects of any single agent that if used alone would need higher doses to be efficacious. The Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend the combination of a CNI, an antiproliferative agent and corticosteroids. Since the risk for acute rejection is highest in the first 3-6 months after transplantation, higher immunosuppressive doses are used in this early period and then immunosuppression is carefully reduced in stable patients to minimize toxicity. CNI block the T-cell receptor early downstream signaling after interaction with the major histocompatibility complex (MHC): peptide complex presented on the surface of the antigen-presenting cell (APC) (signal 1, **Figure 1**). Among the CNI, Tacrolimus (FK) is currently preferentially used as compared to

Cyclosporine (CsA) in many transplantation programs. Anti-metabolites agents such as Mycophenolate mofetil (MMF), Mycophenolic acid (MPA) or Azathioprine (AZA) act on the cell cycle and proliferation. Prednisone is also part of initial immunosuppression but owing to the known adverse effects in the long term, the drug is progressively tapered or even stopped at 1 year in low immunological risk recipients. Mammalian target-of-rapamycin inhibitors (mTORi), such as Sirolimus and Everolimus, act on cell proliferation and are used either as replacement for CNI or combined with low-dose CNI to limit nephrotoxicity.

As immunosuppressive therapy is a lifelong treatment in transplanted patients, research is constantly comparing the different protocols in order to propose greater efficacy in preventing rejection with as little as possible toxicity.

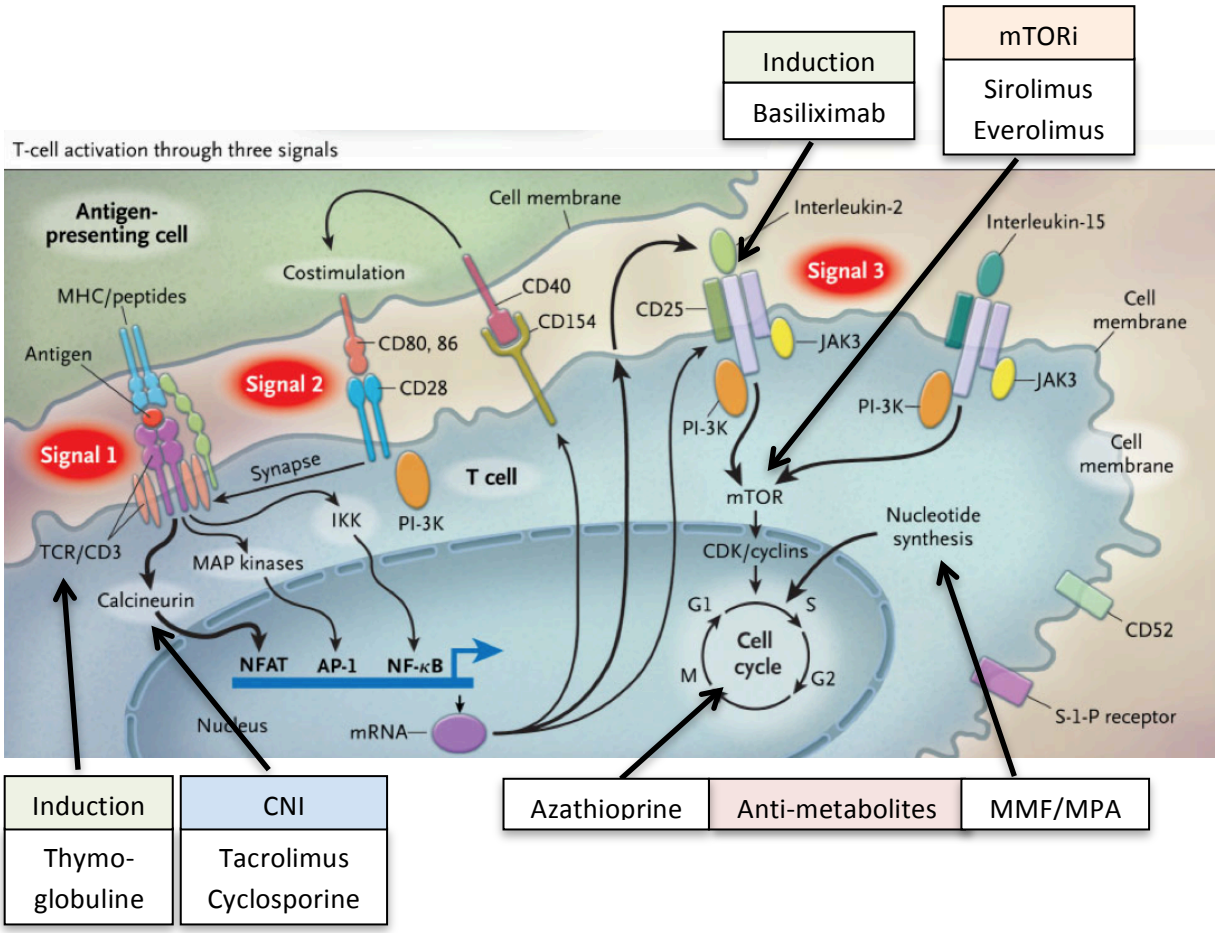


Figure 1. Targets of immunosuppressive drugs used in transplantation. Adapted from Halloran PF. N Engl J Med 2004; 351; 2715.

3. Surgery

The kidney allograft is placed in the iliac fossa and the renal artery and vena are anastomosed on the recipient's iliac external artery and vena. For the ureteroneocystostomy, the bladder is filled with NaCl and the side is opened plan by plan to perform the anastomosis. To rule out an eventual leakage, the bladder is expanded by NaCl and an "anti-reflux" stitch is done on the anastomosis (Lich-Gregoir technique).

The ureteral anastomosis during the kidney transplantation procedure is a common cause (5-9%) (1) of urinary complications such as leakage, ureteral necrosis, stenosis, obstruction, hematuria, vesicoureteral reflux and infections. Despite the improvement of urological techniques, it remains a potential cause of reoperation and postoperative morbidity. The use of an **ureteral stent**, also named double pigtail or double J stent, at the time of the anastomosis has be shown in a meta analysis in 2004 (1) to reduce urological complications from 9% to 1.5%. The proposed benefits of the stent are the continuous decompression of the ureter to avoid anastomotic tension, maintenance of the ureter in a more linear alignment to avoid kinking, and protection from postoperative lumen obstruction due to edema or external compression. Some transplantation centers use the ureteral stent on a routine basis (Geneva, Zurich, Bern), whereas some others use it selectively according to the transplant-surgeon's decision. A small bladder, previous urological operations, double, fine or injured ureters are some reported causes of selective stent placement at the time of the transplantation. Ureteral stents are also used to repair a leakage or a stenosis in the first months after transplantation. There is no consensus regarding the ideal time-interval between the stent placement and ablation. Most of the stents are removed between 1 and 12 weeks after transplantation, once the anastomosis is thought to have healed. Some studies have reported that reducing the stenting time can reduce the complications of the prolonged use of the stent without compromising the benefit. Indeed, the ureteral stent is rapidly covered with a bacterial biofilm when in contact with urine, which can lead to urinary tract infections in immunocompromised kidney transplant recipients. Other complications are migration of the stent, irritation of the bladder resulting in pain, hematuria, stones incrustation and fibrosis of forgotten stents making the removal difficult and at risk of injury. The placement of a stent leads to the need of a postoperative procedure for stent removal by cystoscopy. Another potential complication of ureteral stents is the reported increased risk of BK viremia and BK nephropathy (2)(3)(4)(5) in kidney transplant recipients.

4. BK virus

Historic: The BK virus has been first isolated by Gardner *et al.* in 1971 from the urine of a renal allograft recipient with ureteric obstruction and named after this patient (6). In 1995, Purighalla *et al.* described kidney biopsies of a transplanted man who underwent two reversible episodes of acute rejections and presented thereafter another episode of allograft dysfunction (7). At that time, the kidney biopsy showed a combination of rejection and viral infection with mixed inflammatory interstitial infiltrates, focal tubulitis and enlarged basophilic nuclei of the tubular epithelial cells which reacted with an anti-Simian virus (SV40). Partial clinical response was obtained with steroid treatment and 7-days IvIg treatment. Their attempt to lower

immunosuppression to eradicate the BK viral infection resulted in graft loss. This first description of BKV nephropathy illustrated the 2 main problems of BK infection: the diagnosis was difficult to set as graft dysfunction was first considered as a rejection episode and treated accordingly with increased immunosuppression which lowered the chance to clear the virus. The second challenge was the treatment of BKV nephropathy (BKVN) after diagnosis, which is still nowadays relevant. Indeed, despite numerous attempts, no specific treatment has been found and the reduction of immunosuppression remains the best option, with however a significant risk of graft rejection.

In the past 20 years, the increasing awareness of BK virus in kidney transplant recipients may be in part explained by the earlier detection of the virus in plasma and the apparition of potent immunosuppressive drugs (Tacrolimus and MMF in 1994 and 1995, respectively). Currently, the prevalence of BK viremia reaches about 10-20% (8)(9) at 1 year post transplantation. The prevalence of BKVN confirmed by kidney biopsy was reported to be about 1-10% of kidney transplant recipients (5)(10)(11) in 2009 with different prevalences according to the centers, most likely reflecting the variable local immunosuppression protocols and diagnostic approaches.

At the end of the 1990's, BKVN lead in 30 to 60% of the cases to irreversible kidney graft failure (12). Since about 10 years, the earlier detection of BK viremia and the prompt adaptation of immunosuppression have resulted in the reduction of the incidence of BKVN and kidney graft lost.

Characteristics of BK virus: BK virus is a circular, double-stranded DNA virus from the polyomavirus family that also includes SV40 and JC virus. The genome encodes 3 viral capsid protein (VP1, 2 and 3) and large-T and small-t antigens, which are recognized by our cellular immunity. The BK virus has been divided into 6 genotypes and further subgroups with a specific geographical distribution pattern (13).

Pathogenesis of BKV infection: Exposure to BK virus occurs mostly in childhood as seropositivity reaches 80-90% in young adults around the world and seems not to have changed since the first discovery of the virus in the 1970s. The virus, which infects the urothelial epithelium and renal tubular epithelial cells, remains **latent** and asymptomatic in immunocompetent adults, although 5-10% present intermittent reactivation and low-level viruria (3)(11). In the state of immunosuppression, such as after kidney transplantation, HIV infection or pregnancy, BK virus can reactivate and replicate, causing renal tubular cell lysis with shedding of virus into the urine (**viruria**). About 30% (9) of kidney transplant recipients were described to present viruria, detected by DNA PCR or by the presence of so called "decoy cells" in the urine. These cells with viral inclusions, stained by the Papanicolaou method in cytology, are the sign of BK virus replication in the urinary tract but are not a specific marker of BKV nephropathy. In about 1/3 of the patients with viruria, the BK virus can multiply in the renal interstitium and cross into the peritubular capillaries, causing **viremia**. The presence of BK virus in patient's plasma is detected by DNA PCR, which has become in recent years a widely used method for the early detection of BK virus replication. In some permissive situations, the virus invades the renal allograft, leading to tubulointerstitial lesions, inflammation and interstitial fibrosis.

Polyomavirus-associated (PVAN) or **BK virus nephropathy** (BKVN) occurs without specific clinical signs or symptoms, except for the increase of serum creatinine concurrent with increasing BK viremia titers. Kidney transplant biopsy remains the gold standard for the diagnosis of BKVN with specific histological modifications on hematoxylin-eosin and after immunohistochemical staining using the anti-SV40 antibody (cross-reacting with BK). Graft biopsies also help to rule out other potential complications underlying kidney dysfunction, such as cellular or antibody-mediated rejection. Thus, early detection of low level BK viremia in the context of screening protocols or of rising plasma creatinine, as well as prompt reduction of IS would help to prevent long-term damage of the graft and avoid some of the kidney biopsies.

Besides kidney transplantation, BK virus is known to cause hemorrhagic cystitis and BKVN in highly immunosuppressed bone marrow transplant recipients. Some isolated cases of BK viruria and viremia have been recently described in other SOT recipients (heart, lungs, liver). For example, some cases of BKVN occurring in the native kidneys of heart transplant recipients have been reported (14), showing that BK virus infection may enter in the differential diagnosis of worsening kidney function in SOT recipients other than kidneys. The much higher prevalence of BKVN in kidney transplant recipients has be explained by the potentiating role of alloimmune activation in the kidney graft depending on the degree of donor-recipient HLA mismatches (12). Indeed, BKVN is described to develop in the context of IS (first hit) on a predisposed ground (second hit), such as chemical or mechanical insult of the urothelium resulting in local inflammation. Ischemia-reperfusion injuries at the time of transplantation, prior rejection episodes or the presence of stents or catheters in the urinary tract may provide a suitable environment for the replication of BK virus.

Cellular immune responses to BK: Recipient's T cells recognize antigens (large-T antigen and VP1 capside protein) on the BK virus and become effector cells by secreting IFN-γ, which helps to clear the infection. Schematically, a lower number of BK-specific IFN-γ-secreting T lymphocytes has been reported in patients with BKVN and the reduction of IS was followed by an increase of the IFN-γ activity and the resolution of BKVN. Ineffective immune surveillance by host's T lymphocytes has been proposed as one of the potent factors that contributes to the pathogenesis of BKVN (12).

Humoral immunity to BK: As opposed to cytomegalovirus (CMV) virus, pre-transplantation screening for donor/recipient BK serostatus is not a routine procedure in the clinic. However, humoral immunity may play a role in the pathogenesis of BKVN since a donor seropositive and recipient seronegative status (D+/R-) has been reported as a potential risk factor.

BK virus in kidney transplantation: So far, aside from potent IS therapy, no association has been consistently identified as a risk factor for BK viremia and/or nephropathy. This may suggest that several factors contribute to BK virus reactivation and are likely to interact with one another. In the context of highly immunosuppressed patients at risk of graft dysfunction or loss, the detection of multiple risk factors may help to identify patients with higher risk of BK reactivation in order to prevent BKVN.

Potential risk factors for BK viremia and nephropathy. Adapted from Suwelack *et al*. (15)

Immunosuppression	Other risk factors
Induction	**Donor factors**
Thymoglobulin: increased risk of BK infection, higher rate of BK replication	High degree of HLA mismatches, alloimmune activation (stronger IS) (9)
	BK serostatus mismatch: D+R-
Basiliximab: does not appear to affect the risk of BK infection	Prolonged ischemic time, immunological injury, delayed graft function
Maintenance	**Recipient factors**
CNI	Older age
CsA: *in vitro* suppression of BK replication Introduction in the mid 80's: no rise of BKVN	Male gender
	Previous graft loss (stronger IS, Thymoglobulin)
FK: the most potent inhibitor of BKV-specific T cells, increased risk of BKVN	Retransplantation after graft loss due to BKVN
	Pediatric status (seronegative recipient)
Anti-metabolites	**Surgical/medical factors**
MMF: uncertain effect	Ureteral stent (routine or selective use)
FK-MMF combination: appears to create a permissive environment for BK replication (15)	Other catheters in the urinary tract
Corticosteroids	**Viral factors**
i.v. Pulses: significant association with BK replication and BKVN (9)	Capside serotype, caveolin scaffolding
FK-MMF-Prednisone: has been demonstrated prospectively as a greater risk for BK replication and BKVN (8)	Rearrangement of viral control regions
	Replicative fitness

Treatment of BKVN: The aim of the treatment is to clear BK virus to protect kidney function, while avoiding acute and chronic rejection. In the absence of proven polyomavirus-specific antiviral agents, the mainstay of BK viremia/nephropathy treatment remains the reduction of IS. Cidofovir is an antiviral agent that inhibits viral DNA synthesis, but it has nephrotoxic side effects. Leflunomide and its derivative FK778 are pyrimidine synthesis inhibitors that are efficient for the treatment of BKVN but increase the risk of acute rejection and do not appear to present more benefits than IS reduction alone. Ciprofloxacin has also been described (instead of co-trimoxazole prophylaxis) but this may increase the risk of pathogens resistance and

there is no clear proven duration of treatment. According to the current KDIGO guidelines (16), there is no definitive data confirming the effectiveness of any of these agents for either treating or preventing BKVN.

How IS has to be reduced is not yet clearly proven. The actual propositions of treatment are the followings:

- switch from MMF to AZA, or reduction followed by discontinuation of MMF
- switch from FK to CsA, or 25-50% reduction of the CNI dose
- switch to an mTORi-prednisone protocol

The use of IvIg in parallel to IS reduction has shown good results but the efficacy of IvIg alone is not known. Independently of the reduction of IS, careful monitoring of kidney function and BK viremia by DNA PCR are essential. In their paper, Dall and Hariharan (12) propose a control every 2 weeks during 8 weeks then every month until the clearance of BK viremia and the stabilization of kidney function.

Possibility of retransplantation after BKVN: The retransplantation of patients who have lost their kidney graft due to BKVN appears to be possible (17). It seems that there is no need for BK viremia negativation or nephrectomy of the infected graft before undergoing a second transplantation. However, a relative low rate of BK virus replication is recommended before retransplantation, as well as close monitoring of BK viremia and kidney function to detect possible recurrence under IS. In our center, a few patients have been retransplanted after the loss of their kidney graft due to BKVN. At the time of retransplantation, BK viremia was <1000 copies/ml and did not increase during the follow-up.

Aims

The aims of this study were

- to update the database of the CHUV kidney transplant recipients cohort in order to study this population.
- to define the incidence and predictive factors of BK viremia during the first year after transplantation in the CHUV kidney transplant recipients cohort.
- to study the association between the exposure to an ureteral stent at the time of transplantation and BK viremia during the first year after transplantation.

Material and methods

This study analyses retrospectively the clinical data of 330 adult patients who underwent kidney transplantation at the Centre Hospitalier Universitaire Vaudois (CHUV) between November 2003 and December 2012. All patients have had at least one year follow-up at the Centre de Transplantation d'Organes (CTO) and patients under 20 years of age were excluded because they were followed by paediatricians.

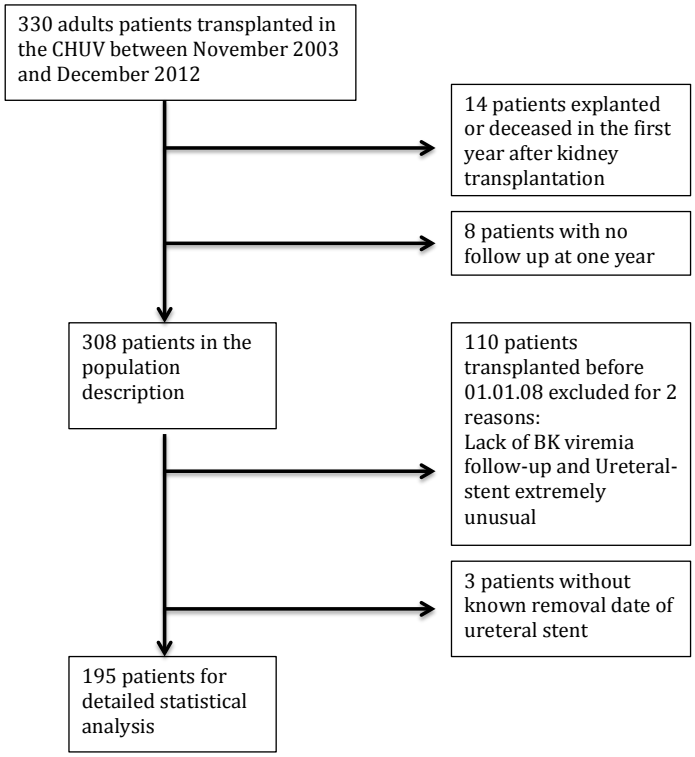
The study is based on data collected on medical paper files and follow-up charts available at CTO and completed by documents archived on electronic centralized files of the CHUV (Archimède). Part of these data have been gathered by Jeremy Jankovic and Mathias Stevanin as part of their Master projects.

The BK viremia results, provided by Professor P. Meylan, as well as the operative protocols, provided by Dr M. Matter, have been analysed and classified for each patient.

The **database** contains the following parameters:

- Baseline recipient's clinical data at the time of transplantation: age, gender, number and type of previous grafts, pre-transplantation kidney disease.
- Type of graft: living vs. deceased donor, transplantation procedure (cold ischemia time).
- Induction and maintenance immunosuppression.
- Clinical follow-up at one year: patient's weight and kidney function.
- BK viremia analysis: first significant positive value (>1'000 copies), peak value, date of viremia negativation (<1'000 copies), kidney biopsy results when available.
- Operative protocols: reason and use of an ureteral stent, date and reason for the ablation of the stent.

Diagram 1. Selection of patients



As depicted in **Diagram 1**, from the 330 consecutive kidney transplantations performed at the CHUV between November 2003 and December 2012, 14 (4,2%) patients were explanted or died with a functioning graft in the first year after transplantation. Eight patients have also been excluded from the study due to no follow-up at one year. The first description of the population in this study has been made on 308 patients, to give a general view of the kidney transplant recipients routinely followed at the CHUV.

As we needed a regular screening for BK viremia and the use of ureteral stent, patients transplanted before 01.01.2008 have been excluded from the detailed statistical analysis. Indeed, either BK viremia screening was inconsistent or the surgeon's use of ureteral stents very occasional before this date. A further 3 patients have been excluded because of no known date of ureteral stent removal. The detailed statistical analysis is finally based on 195 adult patients with at least one year follow-up, including regular BK viremia screening and the possible use of an ureteral stent according to operative findings and surgeon's preference.

Immunosuppression protocol: The induction therapy prescribed at the CHUV was based on Basiliximab and/or Thymoglobulin according to the immunological risk of the recipient. Maintenance therapy generally consisted of the combination of a CNI (FK or CsA), an anti-metabolite (MMF/MPA or AZA) and Prednisone (P). At the CHUV, the standard maintenance immunosuppressive protocol was based on FK, MMF and Prednisone during the first year after transplantation. The CNI doses were adjusted according to therapeutic drug monitoring (TDM), anti-metabolites according to digestive and haematological tolerability, and prednisone following a tapering protocol during the first year.

Prophylaxis of infections: Patients received valgancyclovir (Valcyte) as cytomegalovirus (CMV) prophylaxis during 3 to 6 month according to the donor/recipient (D/R) serostatus. As the glomerular filtration rate (GFR) is often <60ml/min/1.73m^2 after kidney transplantation, the dose was adjusted to it. The patients whose CMV serostatus was D-/R- received valacyclovir (Valtrex) instead as prophylaxis against herpes simplex virus (HSV). All patients were also under antibacterial prophylaxis with co-trimoxazole (trimethoprime-sulfamethoxazole, Bactrim Forte) during 6 months, and prophylaxis against mucosal mycosis by nystatine (Mycostatine) for the first 2 weeks after transplantation.

BK viremia screening: All patients have been regularly screened for BK virus within the first year after transplantation (at 3-6-12 months or 2-4-6-12 months, as per protocol) since 2008. Unless a positive history of BK viremia, no screening (viremia, D/R serology) was performed prior to transplantation. BK virus DNA detection in the patient's serum was performed using quantitative polymerase chain reaction (PCR). The serum samples were analysed in Basel until 30.06.2008, thereafter at the CHUV. No significant difference has been found between the analyses performed in the two different institutions.

For our study, patients had to have at least two consecutive serum samples with BK viremia >1'000 copies/ml to be considered as BK viremia positive. BKVN was defined either by typical histological features on graft biopsies or in the setting of persistent high viremia (>10'000 copies/ml twice).

There are currently no approved guidelines to treat BK viremia/nephropathy. According to CHUV's protocols, immunosuppression was reduced, starting with the dosage of anti-metabolites together with reduced trough levels of FK. If insufficient, anti-metabolites were completely stopped and FK switched to CsA, while steroids were maintained. In some instances, IvIg was used (1 cycle of 3 days for a total dose of 2g/kg weight; in some patients repeated at 1 month interval).

Statistical analysis: Data were gathered in a Microsoft Office Excel database, before being simplified and grouped into categories. In a first phase, descriptive statistics were performed using all the patients who were included in the study, in order to define general characteristics of the population transplanted and regularly followed at the CHUV. We then analyzed and compared patients based on their BK status and the presence/absence of a ureteral stent.

Statistical analyses were performed using R-software, with the help of Dr I. Salvade. Continuous data were expressed using mean values (standard deviation) and categorical data were described as numbers (percentages). Categorical data including demographic, clinical events (ureteral stent), immunosuppressive drugs and BK-related events were compared using Fisher's exact test. Associations between the presence of a ureteral stent as well as other risk factors and the occurrence of BK viremia was explored by univariate and multivariate analysis. A p-value of <0.05 was considered as statistically significant.

Results and discussion

1. Donors and recipients characteristics

Table 1. Description of the study population				
	Total population 2003-2012 (n=308)		**Subpopulation 2008-2012** (n=195)	
	n	**%**	**n**	**%**
Gender F / M	92/216	29.9/71.1	55/140	28.2/71.8
Mean age (years)	51.7		52.9	
First / prior Tx	239/69	77.6/22.4	152/43	77.9/22.1
Living / cadaveric donor	151/157	49.0/51.0	101/94	51.8/48.2

Table 1 shows in parallel the characteristics of the 308 patients transplanted with a kidney at the CHUV between November 2003 and December 2012, as well as of the subpopulation of 195 patients transplanted between 2008 and 2012 that we analysed in more detail for BK viremia. There was no significant difference in gender distribution, mean age at transplantation, type of donor and immunological risk of the recipient (low i.e. first graft vs. high immunological risk i.e. more than 1 transplantation) between the 2 groups. This reflected a similar starting population at transplantation in the two periods. Regarding post-transplant management, BK viremia screening was introduced systematically only in the newest era.

About 71% of the transplanted patients were men, a proportion that can also be found in other studies about kidney transplantation (2)(3). As we only analysed the adult population, the age range extended from 21 to 79 years, with a mean age of 52 years. Around 22% of the population had already received a prior transplant (kidney, heart, liver or lungs) and was therefore considered at high immunological risk. This is important information as the immunological risk of the recipient dictates the intensity of the induction and maintenance immunosuppressive therapy after transplantation.

2. Immunosuppression

Table 2. Immunosuppression					
		Total population 2003-2012 (n=308)		**Subpopulation 2008-2012** (n=195)	
		n	**%**	**n**	**%**
Induction	Basiliximab	210	**68.2%**	134	**68.7%**
	Basiliximab + Thymoglobulin	38	12.3	27	13.8
	Thymoglobulin (3-4 days)	38	12.3	17	8.7
	Thymoglobulin >4 days	8	2.6	6	3.1
	Thymoglobulin + IvIg	14	4.5	11	5.6
Maintenance	FK / CsA	295/13	95.8/4.2	187/8	95.9/4.1
	MMF / AZA	294/14	95.5/4.5	185/10	94.9/5.1
	FK + MMF + P	281	**91.2%**	177	**90.8%**
	Other associations	27	8.8	18	9.2
Treatment at one year	FK / CsA / mTORi	282/21/5	91.6/6.8/1.6	180/13/2	92.3/6.7/1.0
	MMF / AZA / None	262/25/ 21	85.1/8.1/6.8	165/17/ 13	84.6/8.7/6.7
	FK + MMF ± P	242	**78.6%**	153	**78.5%**
	Other associations	66	21.4	42	21.5

Table 2 shows that at the time of transplantation, the standard immunosuppression treatment consisted mainly of **Basiliximab-based induction** followed by the association of FK, MMF and Prednisone as maintenance therapy. Basiliximab induction therapy (2 doses, day 0 and 4) was given to low immunological risk patients (first transplantation, non-sensitized), whereas the high immunological risk patients (≥ 2 transplantations, prior HLA immunization) received Thymoglobulin instead. In case of delayed graft function (DGF), Thymoglobulin was added to Basiliximab given on day 0, or Thymoglobulin treatment was prolonged (>4 days) in order to delay the introduction of potentially nephrotoxic CNI (FK or CsA), until the recovery of acute tubular necrosis and renal function. About 5% of the recipients were hypersensitized with pre-existing donor-specific antibodies (DSA). These patients received Thymoglobulin induction and IvIg to prevent antibody-mediated graft rejection.

The maintenance therapy was initiated during the first days after transplantation and was mainly (>90%) based on the association of FK, MMF and Prednisone (P).

This protocol was relatively standardized and stable during both study periods, (whole period 2003-2012, and subpopulation studied between 2008-2012 for BK events). For about 4% of the population, CsA has been preferred to FK due to the risk of post-transplantation diabetes, chronic HCV or depending on the underlying nephropathy (for example in the case of primary focal segmental glomerulosclerosis). For another average 4.5% of the population, MPA-based agents (Cellcept® or Myfortic®) were switched to AZA in the early post-transplant period, mainly because of gastrointestinal side effects.

The IS therapy at 1 year after transplantation, which often reflects the immunological and infectious events that had occurred during the year, was still mainly represented by the association of FK, MMF ± Prednisone (78%). As compared to the early IS maintenance therapy, the percentage of patients under CsA (vs. FK) or AZA (vs. MPA-based agents) was slightly increased. In less than 2% of patients, mTORi were used, usually to replace CNI in case of severe nephrotoxicity and/or thrombotic microangiopathy. Some patients were on CNI (either FK or CsA) and Prednisone, but received no anti-metabolites (MMF or AZA). This was usually the case if patients had high levels of BK viremia and/or BKVN during the first year. Prednisone was tapered progressively during the first year and sometimes stopped before month 12 after transplantation, usually leaving patients under FK and MMF therapy.

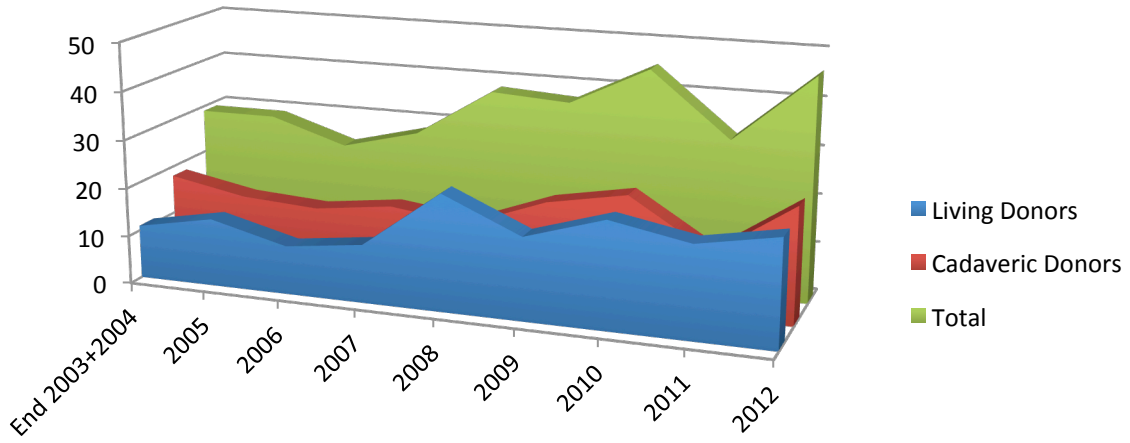
3. Kidney transplantation characteristics

Table 3. Kidney transplantation

	Total	Living donors		Cadaveric donors	
	n	n	%	n	%
End 2003+2004	**29**	11	**37.9**	18	**62.1**
2005	29	14	48.3	15	51.7
2006	24	10	41.7	14	58.3
2007	28	12	42.9	16	57.1
2008	38	24	63.2	14	16.8
2009	**37**	17	**45.9**	20	**54.1**
2010	45	22	48.9	23	51.1
2011	32	19	59.4	13	40.6
2012	**46**	22	**47.8**	24	**52.2**
Total	**308***	**151**	**49.0%**	**157**	**51.0%**

*14 patients explanted or deceased in the first year post-transplantation and 8 with no follow-up at one year are not represented in this table.

Graph 1. Kidney transplantations performed at CHUV



As shown in **Table 3 and Graph 1**, the number of renal transplantations performed at the CHUV has increased through the study period (about +50% in 9 years). On average, the proportion of living donors has also increased, with some variations according to the years. Because of the aging of the population and the progresses in medical and surgical management of patients suffering from chronic kidney diseases, more patients are becoming suitable candidates for transplantation, extending the waiting list. However, the number of cadaveric donors remains insufficient. The increasing awareness of the possibility of kidney donation by a relative or partner/friend together with progresses in minimal invasive surgical techniques for kidney removal have increased the proportion of living donation in recent years. But the gap between the number of patients on the waiting list and kidney transplantations performed every year is still growing. In 2014, according to Swiss Transplant statistics, 1062 patients were waiting for a kidney while a total of 296 have been transplanted in Switzerland (https://www.swisstransplant.org).

4. Surgical use of ureteral stents at the time of kidney transplantation

Table 4. Description of the use of ureteral stents		
Total patients transplanted between 01.01.08 and 31.12.12 (n=195)		
	n	**%**
Stent / no stent	**76** / 119	**39%** / 61%
Causes of stent placement (n=76)		
Ureter	18	23.7%
Short/fine ureter	15	
Double ureter system	3	
Vascular	14	18.4%
Inferior polar artery	9	
Devascularized ureter or with little adjacent fat	5	
Bladder	5	6.6%
Micro-bladder (anuric or post radiotherapy)	3	
Anastomosis to an ileal bladder (Bricker)	2	
Anastomosis	8	10.5%
Unsatisfactory surgical anastomosis	2	
Ureteral leak	1	
Stenosis	5	
No specific cause reported	31	40.8%
Causes of stent ablation (n=76)		
Per protocol (no problem reported)	57	75.0%
Infection	9	11.8%
Discomfort/intolerance/displacement	7	9.2%
Hematuria (micro/macro)	2	2.6%
Obstruction	1	1.3%
Time interval between stent placement and ablation (n=76)		
<1 month (<30 days)	19	25.0%
1-2 months (31-60 days)	**39**	**51.3%**
2-3 months (61-90 days)	13	17.1%
>3 months (>90 days)	5	6.6%

To analyse the potential effect of a ureteral stent on the incidence of BK viremia after kidney transplantation, the surgery protocols were reviewed and all information about ureteral stents were recorded. From the 195 patients transplanted between 01.01.08 and 31.12.12, a ureteral stent was used in 76 cases (39%). At our institution, during the study period, the decision to place a ureteral stent was made by the transplant surgeon at the time of transplantation.

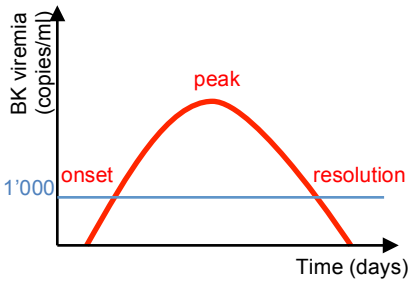
We analysed the reasons of stent placement and classified them into 5 categories (**Table 4**). Eighteen (23.7%) stents have been placed because of ureteral problems (short/fine ureter or double ureter system). Inferior polar artery, devascularized ureter or little remaining adjacent fat were reported as the cause of 14 stents placements (18.4%). Five stents (6.6%) were placed because of a specific bladder anatomy (micro-bladder or ileal bladder). Complications of the anastomosis at the time of transplantation, an unsatisfactory surgical anastomosis and subsequent early ureteral leak or stenosis resulted in 8 stents (10.5%). For the other 40% of ureteral stent placement, no reason was reported in the operative protocol and these stents have probably been placed according to the surgeon's preference.

Regarding stent ablation, 75% of them have been removed per protocol, while no problem was reported. Nine stents (11.8%) have been taken out because of urinary tract infection, seven (9.2%) because of discomfort, intolerance or displacement of the stent, two (2.6%) because of haematuria and one (1.3%) following an obstruction. While most ureteral stents have been used without complication, our analysis illustrates that it is however not an insignificant procedure as reflected by 24.9% of the patients that have suffered from complications. Furthermore, the removal of ureteral stents requires an additional urological procedure, which can be uncomfortable for the patient.

According to our protocol, ureteral stents had to be removed between 4 to 6 weeks post transplantation. That was the case for more than 50% of the patients and in this group only 4/39 stents were removed following a complication. Nineteen stents (25%) were removed before 4 weeks and for 2/3 of them (12/19) an early complication was reported. Thirteen stents (17.1%) stayed in place an extra month and 5 (6.6%) more than 3 months. In these two last groups, only 3 episodes of urinary tract infections were reported as a complication. Among the 5 stents that stayed more than 3 months, 3 had been placed subsequent to an anastomosis stenosis. Of note, 1/5 of these latter patients presented transient (<1 month) early BK viremia overlapping the stenting period, but resolution of viremia occurred without the need of stent removal.

5. Incidence of BK viremia after kidney transplantation

Table 5. Description of BK viremia				
Total patients transplanted between 01.01.08 and 31.12.12 (n=195)				
	n	**%**		
BK viremia/ no BK viremia	**43**/152	**22**/78		
BK viremia in the 1st year post-transplantation	**37**	**19%**		
Peak BK virus copies/ml in plasma PCR (within the first year post Tx) (n=37)				
1'000-10'000	9	24.3		
10'000-100'000	20	54.1		
>100'000	8	21.6		
Time distribution	BK viremia **onset** (n=43)		BK viremia **peak** (n=43)	
1st month: 1-30 days	0	0	0	0
2nd month: 30-60 days	5	11.6	1	2.3
3rd month: 60-90 days	11	**25.6%**	4	9.3
4th month: 90-120 days	12	**27.9%**	18	**41.9%**
5th month: 120-150 days	4	9.3	5	11.6
6th month: 150-180 days	3	7.0	5	11.6
7-12th month: 180-365 days	2	4.6	4	9.3
> 365 days	6	14.0	6	14.0
Time interval between	**Onset** and **peak** of viremia (n=43)		**Onset** and **resolution** of viremia (n=43)	
0 days (beginning value=peak)	12	27.9	-	-
1-30 days	22	**51.2%**	3	7.0
30-60 days	7	16.3	15	**34.9%**
60-90 days	2	4.6	8	18.6
90-180 days	0	0	6	14.0
180-365 days	0	0	5	11.6
> 365 days	0	0	6	14.0



Of the 195 patients transplanted between 01.01.08 and 31.12.12, BK viremia >1'000 DNA copies/ml was found in 43 patients (22%), of which 37 (19%) within the first year after transplantation (**Table 5**). When looking at the time distribution of BK viremia onset, most events occurred during the 3rd (60-90 days) and 4th (90-120 days) month after transplantation, respectively 25.6% and 27.9%. No BK viremia was detected during the first month, probably reflecting very rare screening performed during this early period. Only 2 cases (4.6%) were diagnosed between months 7 and 12

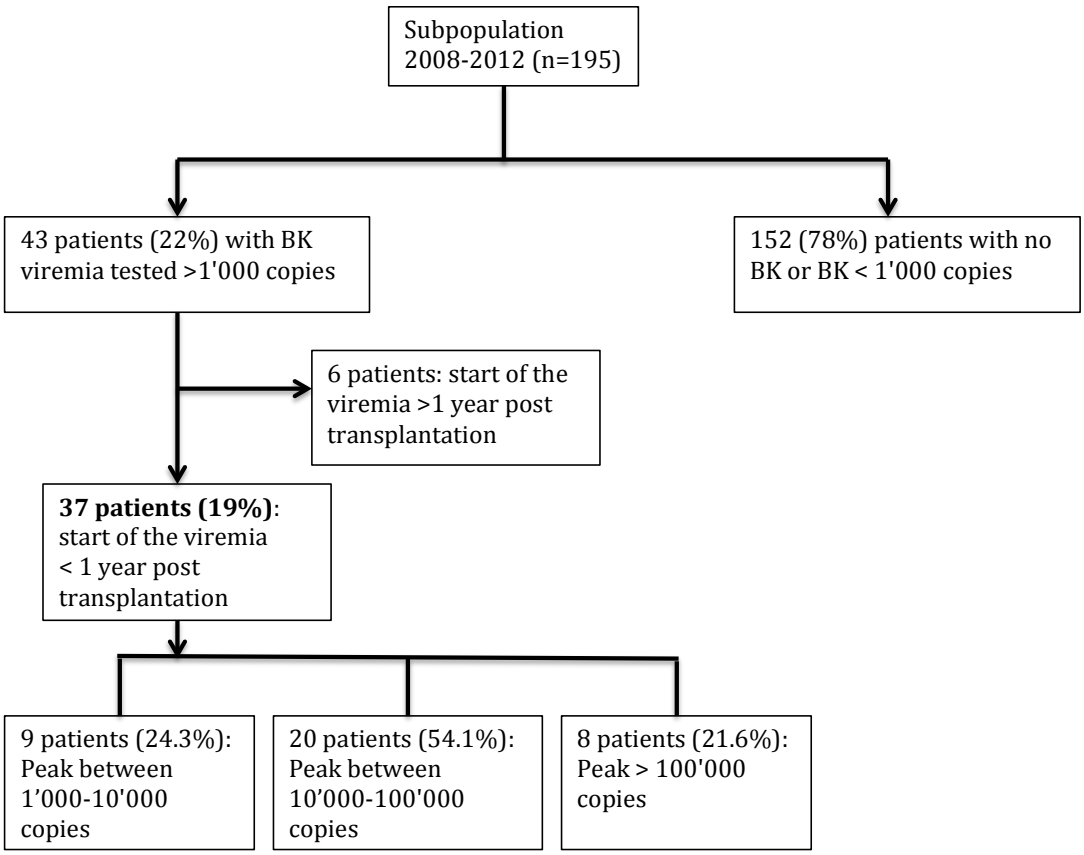
The time distribution of BK viremia onset (>1'000 copies/ml) and peak values that we observed in our study tends to reinforce the current screening strategy performed at the CHUV, i.e. at month 2, 4, 6 and 12 after transplantation, independent of kidney function. Extra testing at month 3 might be proposed as 25.6% of BK viremia started at that time. Interestingly, 14% of BK viremia cases were newly diagnosed after 12 months: 5/6 during the second year post transplantation and 1/6 during the third year. The KDIGO guidelines report that 95% of BKVN occur in the first 2 years after kidney transplantation (16). Beyond the first year after transplantation, there is no clear guidelines regarding whether and at which frequency screening should be performed in the absence of previous BK viremia. The current clinical practice at the CHUV is to perform screening in the case of kidney dysfunction and/or intensification of the immunosuppressive regimen.

We defined the peak of BK viremia as the highest values of copies/ml measured in a patient during the 1st year follow-up. More than 50% peak values happened during the first month after onset of BK viremia and 16.3% during the second month. This may reflect rapid replication of the virus in peripheral blood after onset, and the need for rapid and early detection to prevent BKVN. For 27.9% of recipients, their first BK viremia result was already the peak value. Prompt management with reduction of immunosuppression probably accounts for the rapid decrease of BK viremia that we observed after the first detection. As the incidence of BKVN has been linked to the extent of BK viremia, we stratified peak values within the first year into 3 categories: 1'000 to 10'000 copies, 10'000 to 100'000 copies and >100'000 copies, with 24.3%, 54.1% and 21.6% of the patients in the respective groups. Among the 37 patients with positive BK viremia during the first year after transplantation, 28 (75.7%) had peaks >10'000 copies, a cut-off value considered as predictive of intragraft viral replication resulting in tubulointerstitial damage. To put our data into perspective, Kayler *et al.* (2) reported 93/600 (15.5%) BK viremia (defined in their study as >500 copies/ml) within the first post-transplant year and in 70 of these cases (75.3%), the peak BK PCR level was >10'000 copies/ml. In our studied transplanted population, 28 out of total 195 patients (14.4%) had a peak BK viremia >10'000 copies which represents a high positive predictive value for BKVN (KDIGO)(16). In our centre, we did not systematically perform kidney biopsies to confirm the diagnosis of BKVN in these patients, but IS was modified in all cases as if they had BKVN. As a comparison, the incidence of BKVN reported in the literature is much lower (2-5%) (17).

Resolution was defined as the date BK viremia reached a value <1'000 copies/ml, in the absence of further increase in later follow-ups. One third of the patients cleared the virus between 30 and 60 days after the onset of viremia and at 6 months, 74.5% of the patients had reached a value <1'000 copies. The remaining patients that displayed persisting viremia (>one year) had all reached a peak value >10'000 copies/ml, some of them even >100'000 copies/ml.

Diagram 2. Distribution of BK viremia

The following diagram summarises our findings regarding BK viremia incidence.



6. Effect of BK viremia on kidney function at one year

We considered kidney function at one year as one of the outcomes of this study as it would, at least in part, reflect the severity of BKVN in the presence of BK viremia. Kidney function at 1 year after transplantation was also shown to be a good predictor of future graft and patient survival. The glomerular filtration rate was calculated (eGFR) using the Cockroft and Gault formula, with patient data collected at one year (weight, serum creatinine, sex and age). Of note, the Cockroft and Gault formula has been developed for chronic kidney disease (CKD) patients and may not be accurate in the setting of renal transplantation. However, other available formulas (MDRD, CDK-EPI, Nankivell) that are used in CKD patients have also not been validated to estimate GFR after kidney transplantation.

Table 6a. Kidney function at 1 year	
	eGFR (ml/min)
Mean kidney function (n=195)	65
Patients with **no BK viremia** (n=158, 81%)	**67**
Patients with **BK viremia** (n=37, 19%)	**58**
BK viremia 1'000-10'000 copies/ml (n=9)	65
BK viremia 10'000-100'000 copies/ml (n=20)	61
BK viremia >100'000 copies/ml (n=8)	44

The mean eGFR at 1 year after kidney transplantation was 65 ml/min, which corresponds to CKD stage II (**Tables 6a and 6b**). In our population of 195 kidney transplant recipients, we found a significant difference (p=0.019) between the two groups (no BK viremia vs. BK viremia during the first year post transplantation). The eGFR decreased as BK viremia levels increased, **in particular above 10'000 copies/ml**, which could be explained by potential BKVN and associated renal damages.

Table 6b. Kidney function					
CKD stage	**eGFR (ml/min)**	**No BK viremia** (n=158)	**BK viremia (copies/ml)** (n=37)		
			1'000-10'000	10'000-100'000	>100'000
I	≥ 90	15 (9.5%)	1	0	0
II	60-89	82 (51.9%)	4	12	1
III	30-59	58 (36.7%)	4	8	5
IV	15-29	3 (1.9%)	0	0	2
V	< 15	0	0	0	0

7. Predictive factors for BK viremia after kidney transplantation

As described before, the population analyzed in our study was demographically representative of current adult kidney transplant recipients followed in most centers, with a mean age of 52.9 years (range 21-79). Of note, 71.8% of our patients were men. We first performed univariate analysis to determine the association between the use of **a ureteral stent** at the time of initial surgery and BK viremia. The Fisher's Exact Test did not show a significant association (p=0,38). This result should however be interpreted with caution as we had relatively few events: 43 BK viremia in the population studied (22%) and only 76 out of the total 195 patients (39%) had a ureteral stent. Other studies have shown an association between the use of an ureteral stent and the occurrence of BK viremia (2)(3)(4)(5)(8). As for our study, these were all retrospective and single-center studies, with the number of patients ranging from 66 to 621 (**Table 7**). However, the rate of ureteral stents was not comparable between centers (probably reflecting local surgical protocols), as well as the definitions of BK-related outcomes (levels of viremia, occurrence of BKVN).

Therefore, to determine whether the use of a ureteral stent at the time of surgery is an independent risk factor for BK viremia in kidney transplant recipients, prospective randomized studies with careful analysis of concomitant variables (immunosuppression protocols, initial surgical protocols, urological complications during the first year, etc.) are needed.

Table 7. Comparison with available literature

Author, Year Type of study	**Total patients** (n)	**Ureteral Stent** n (%)	**BK viremia** n (%)	**Inferior limit of viremia**	**p-value**	**OR**
Brennan, 2005 Retrospective	200	54 (25%)	23 (11.5%)	10'000 copies/ml	0.018	HR = 3.0
Thomas, 2007 Retrospective	66	31 (47%)	*2% BKVN*	*BKVN (biopsy)*	0.003	Univariate 5.6 Multi 4.71
Siparsky, 2011 Retrospective	186	124 (67%)	32 (17%)	2.7 log copies/ml	0.02	3.17
Kayler, 2012 Retrospective	600	295 (49.2%)	93 (15.5%)	500 copies/ml	Uni 0.06 Multi 0.03	Uni 1.53 Multi 1.65
Hashim, 2014 Retrospective	621	295 (47.5%)	115 (19.0%)	500 copies/ml	Uni 0.05 Multi 0.04	1.55
Our study, 2015 Retrospective	195	76 (39%)	43 (22%)	1000 copies/ml	0.38	

In our routine practice, we had noticed the occurrence of BK viremia in patients presenting **urological complications** in the first year after transplantation, such as benign prostatic hyperplasia, urethral stenosis, urinary retention and ureteroneocystostomy leakage, which required urological manipulations after initial transplantation surgery (vesical catheter, transurethral resection of the prostate, ureteral stent or nephrostomy). As this study was retrospective based on medical charts and electronic files, the quality of the data was not sufficient to study the association between post-operative urological complications and BK viremia. This could be best verified in a prospective study, as previously mentioned.

The hypothesis of **urothelium injury** as a necessary determinant for the development of a BKVN has been made by Atencio *et al.* (18) who proposed a two-hit hypothesis, also reported by Thomas *et al.* (5). The first hit corresponds to the reactivation of BK virus from latency in the setting of immunosuppression. The second hit, which appears to be necessary for de progression of BKVN, is renal cell injury. This is supported by the fact that in murine models, BK virus reactivation could be induced by various chemical and mechanical insults, such as renal artery clamping to produce renal ischemia. This theory could partially explain why BKVN is more frequent in kidney transplant recipients as compared to other immunosuppressed patients. The renal cell insult produced by ischemia-reperfusion and/or rejection episodes in the context of immunosuppression may provide the necessary environment for BK virus replication. In addition, in animal models, ureteral stents have been shown to cause superficial epithelial destruction with erosions, ulcerations and inflammatory reactive changes. Thomas *et al.* (5) postulated that similar reactive urothelial changes could be expected to occur in the donor ureter secondary to the placement of a stent, which may result in BK virus reactivation and replication.

As potent **immunosuppression** is a known risk factor for BK viremia, we then analyzed the role of IS therapy on BK-related events in our transplanted population. Neither differences in the induction (Thymoglobulin vs. Basiliximab) nor in the maintenance therapy (FK vs. CsA) were significant determinants for BK viremia. According to literature, Thymoglobulin, the T-cell depleting agent, increases the risk of BK infection, whereas Basiliximab, the reversible T-cell modulator, does not. FK is known as a major risk factor for BK viremia as it inhibits BKV-specific T cells while CsA has been shown to suppress viral replication *in vitro*. In our population, maintenance protocols were very homogeneous and therefore too little patients were receiving CsA for a statistically relevant comparison. In our study, where >95% of the patients initially received FK and >90% were prescribed FK+MMF+P maintenance therapy early after transplantation, it would have been more informative to compare FK trough levels over time and possible associations with BK viremia. Indeed, the though level, rather than the dosage, represents the global biological effects of IS. It was unfortunately not part of our initial data collection and would have implied statistical analysis beyond the scope of this retrospective study. In recent years, with the improvement of rejection management and the awareness of BKVN among other complications, guidelines have recommended lower target levels of IS. To some extent, the CHUV patients have been managed according to these recommendations, which could in part explain lower incidences of high titer BK viremia (>10'000 copies/ml) and BKVN.

Since **ischemia-reperfusion** is known to cause initial graft damage, we compared BK viremia in kidney transplant recipients of living vs. cadaveric donors. In our population of patients transplanted between 2008 and 2012, the two groups were comparable in size, with 101 (51.8%) and 94 (48.2%) grafts from living and cadaveric donors, respectively. We found no significant association between the type of graft and BK viremia. Some studies mention prolonged ischemia time as a risk factor for BK viremia. Compared to the USA, Switzerland is a small country and cold ischemia time is usually much shorter (living donors: 42min-4h, cadaveric donors: mean 10h15, range 4h30-19h30).

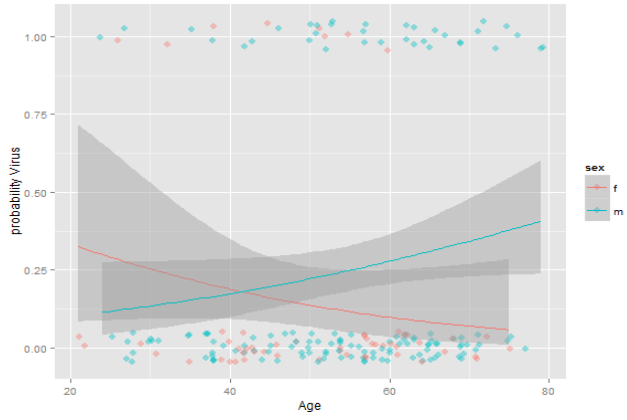


Figure 2. Probability of BK viremia, according to age and gender (F, red line; M, blue line).

Concerning the **demographic characteristics** of our population, recipient's age and sex, analyzed separately, were no predictive factors for BK viremia. But by combining these two variables, we observed that the probability for a man to suffer from BK viremia increased with age, with a p-value reaching significance (p=0.05) (**Figure 2**). In comparison, the risk did not increase with age in women. As mentioned before, urological complications, which are more frequent in older men, could be a contributing factor for BK virus replication.

Conclusion and perspectives

In the past 20 years, BK virus has emerged as a cause of graft dysfunction after kidney transplantation, leading to specific measures in the post-transplantation management. Indeed, the importance of early detection of BK viremia in plasma by DNA PCR in order to adapt IS and prevent BKVN is now widely acknowledged (16). So far, besides the amount and type of IS, little has been published on other risk factors and current data are controversial.

The first aim of our study was to define the incidence and kinetics of BK viremia in our recipients of kidney grafts during the first year. Among the 195 patients transplanted at the CHUV between 01.01.2008 and 31.12.2012, 37 (19%) presented a BK viremia >1'000 copies/ml, which is comparable to published data (2)(3)(4). In most cases, the onset of BK viremia occurred early after transplantation. In 65.1% of the cases, positive BK viremia was detected during the first 4 months (defined in our study as BK viremia >1'000 copies/ml in at least two consecutive serum samples), with a peak viremia value at 30 days following onset in 51.2%. Interestingly, 6 patients were newly diagnosed after 12 months of transplantation. Prompt management with reduction of IS was generally set up in order to reduce viral loads and to protect the graft. To assess the consequences of BK viremia, we analysed kidney function at one year and observed a significant difference (9 ml/min, p=0.019) between the mean eGFR of patients with no BK viremia (eGFR 67 ml/min) and patients with BK viremia (eGFR 58 ml/min). The eGFR decreased as BK viremia levels increased, possibly reflecting BKVN-associated damages.

Considering the total incidence of BK viremia in our population (22%), the fact that BKVN is a factor of poor prognosis for graft function and that viremia detection by PCR allows early diagnosis and management, our data reinforce the importance of regular screening after kidney transplantation. The KDIGO guidelines (16) propose a monthly screening schedule for the first 3-6 months after transplantation, then every 3 months until the end of the first post-transplant year, as well as whenever there is an unexplained rise in serum creatinine and after treatment for acute rejection. We could propose adding to the current CHUV protocol (PCR at 2-4-6-12 months then 1x/year) extra testing at 3 and 5 months and after 12 months in the presence of an unexplained rise in serum creatinine.

In order to identify the population at risk for BK virus reactivation and to propose a more individualised follow-up, in particular regarding screening and the degree of IS, we analysed potential factors and their association with the occurrence of BK viremia in our population. We found no significant association with the type of graft (living vs. cadaveric donor), or with the IS protocol (Basiliximab vs. Thymoglobuline, FK vs. CsA). However, by combining recipient's age and gender, it appeared that older men had a higher risk to reactivate the BK virus, with a p-value reaching significance (p=0.05). This finding needs to be confirmed in further studies with a special attention to urological complications and manipulations, which could not be analysed in detail in our retrospective study.

The placing of a ureteral stent has been mentioned in the literature as a potential risk factor for BK viremia (2)(3)(4)(5)(8). In our transplanted population, we reported the use of ureteral stents in 76/195 patients (39%). The decision was made by the transplant surgeon, according to the ureter and bladder anatomy or due to

unsatisfactory vascularisation or anastomosis complications. In our study, we found no significant association between the use of a ureteral stent and BK viremia.

Overall, this study is limited by its design as a retrospective single-centre cohort analysis, based on medical paper charts and electronic files in which not all outcome data were strictly reported (for instance, urological complications). Although we initially aimed to include all transplanted patients from end of 2003 onwards (n=308), the study was finally restricted to a more recent era (2008 onwards, n=195) when BK viremia screening protocols were applied at CHUV as well as more frequent use of ureteral stents. Moreover, in recent years in our centre, BKVN was an assumed diagnosis in the setting of persistent high BK viremia i.e. >10'000 copies/ml at least twice, a cut-off value considered as predictive of intragraft viral replication and tubulointerstitial damage. Thus, kidney biopsies were not systematically performed to confirm the diagnosis and evaluate the severity of nephropathy.

BK virus, first detected in 1971 and considered as a cause of kidney graft dysfunction since 1995, remains a fascinating entity with lots of remaining unanswered questions. The aetiology of BK replication and BKVN, which seems to be multifactorial, has to be better defined in order to optimise the follow-up of kidney transplant recipients. So far, in the absence of proven polyomavirus-specific antiviral agents, the mainstay of BK viremia/BKVN management remains the reduction of IS, which has to be carefully monitored to avoid acute and chronic rejection. The early detection of BK viremia prior to graft damage appears nowadays as the best clinical option to improve graft outcome after kidney transplantation. The advent of specific anti-viral therapies or of more standardised protocols aiming at reducing IS in the case of BK viremia would further help the clinician minimize early graft dysfunction and loss.

Based on current knowledge and on our data, we could suggest performing a prospective randomized multicenter study with controlled cofactors (immunosuppression, surgical use or not of ureteral stents) and detailed follow-up charts (including urological complications/manipulations) to better analyse the determinants of BK viremia and BKVN. Interestingly, in Switzerland some transplantation centers (Geneva, Bern, Zurich) use systematically ureteral stents at the time of transplantation surgery. It would be interesting to analyse BK viremia rates during the first year in these different centers and to compare the data with our study. In this regard, the data gathered prospectively since 2008 in the multicenter Swiss Transplant Cohort Study (STCS) could help answer these questions.

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