BK Virus Infection: an Update on Diagnosis and Treatment

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Case Discussion

- 62 year old African-American male; blood group A and PRA 0%
- ESRD-etiology not clear- on dialysis since June 2007
- DD kidney transplant on 4/11/2010
- Delayed graft function-dialysis for 2 weeks; induction with rATG (3 doses)
- Maintenance with tacrolimus + mycophenolic acid + prednisone
Case Discussion

- Scr is down to 1.86 mg/dL at 4 weeks posttransplant.
- Scr is up to 2.7 mg/dL 11 weeks posttransplant.
- Blood BK viral load 4.3 log copies/mL at the time of the biopsy-increased to 5.6 log copies/mL 4 weeks later.
Case Discussion

• Biopsy: consistent with BK nephropathy
  • Viral cytopathic effect-intranuclear inclusion bodies
  • Degenerative tubular epithelial changes with sloughing and cellular and granular casts
  • Variable interstitial nephritis
BK Virus

1971: described by Gardner SD and colleagues in Lancet: “isolated from urine after renal transplantation”-named BK virus (the initials of the patient who had ureteric stenosis)

1983: first report on tubulointerstitial nephritis (6 year old boy with primary immune deficiency)

Not truly appreciated until mid 1990’s

Rosen S, et al. NEJM 1983
Kaplan-Meier estimated incidence of treatment of BK, 2003 to 2006 OPTN database (n=48,292): rising over time-0.7% at 6 months posttransplant; 2.18% at 1 year, 3.45% at 2 years, and 6.6% at 5 years; higher risk of subsequent graft loss (at least 2 fold)

Higher center volume and living kidney donation: protective; more BK with the use of r-ATG and TAC/MMF combo and more in recent transplant years

Dharnidharka VR, et al. Transplantation 2009
BK Virus

• Belongs to a family of DNA viruses called polyomaviruses - originally Papovavirus - (includes JC virus, KI, WU and SV40 viruses)

• Based on DNA sequence variations, BK virus can be divided into 6 subtypes or genotypes
  • Genotype I - most common (80%), followed by genotype IV (15%)

• Primary Infection: subclinical
  • Childhood nonspecific viral illness - respiratory
BK Virus

- Long-lived latency in uroepithelium and renal tubular cells (not in reticuloendothelial cells-like herpes viruses)
- Antibodies against BKV: 50% of children by age 3, 60-90% by age 10, and 80-90% by age 20
- Uncommon in healthy individuals; Reactivation in immunocompromised patients
Linear Progression of Disease

- Asymptomatic BK viruria (1/3 with high-level viruria develop viremia)
- BK viremia-without intervention 1-10% could progress to BK nephropathy
- Parenchymal damage and progressive deterioration of graft function (BK nephropathy)
- Also hemorrhagic cystitis, ureteral ulceration/stenosis, and progressive multifocal leukoencephalopathy (PML)
### BK Prevalence in Contemporary Studies with Prospective Screening

<table>
<thead>
<tr>
<th>Reference</th>
<th>Decoy cells</th>
<th>BK viruria</th>
<th>BK viremia</th>
<th>BK nephropathy</th>
<th>Graft loss due to BK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hirsch et al. 2002</td>
<td>29%</td>
<td>*</td>
<td>13%</td>
<td>6.4%</td>
<td>0%</td>
</tr>
<tr>
<td>Brennan et al. 2005</td>
<td>35%</td>
<td>11.5%</td>
<td>*</td>
<td></td>
<td>0%</td>
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<tr>
<td>Koukoulaki et al. 2008</td>
<td>25%</td>
<td>14%</td>
<td>*</td>
<td></td>
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</tr>
<tr>
<td>Almeras et al. 2011</td>
<td>*</td>
<td>11%</td>
<td>0.85%</td>
<td>0.85%</td>
<td></td>
</tr>
<tr>
<td>Thakur et al. 2011</td>
<td>15.7%</td>
<td>43%</td>
<td>8%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Sood et al. 2012</td>
<td>17%</td>
<td>27%</td>
<td>2.1%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Hirsch et al. 2013</td>
<td>25.4%</td>
<td>13.7%</td>
<td>*</td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

* Prevalence of event not reported

Sawinski D and Goral S. NDT 2015
Risk Factors for BK Infection

- **Donor risk factors:**
  - BK virus seropositivity
  - Degree of HLA mismatching
  - Absence of HLA C7

- **Recipient risk factors:**
  - Older recipient age, male recipient
  - Recipient race (non African-American)
  - Previous graft loss due to BK nephropathy
  - Presence of diabetes
  - Absence of HLA C7
  - BK virus seronegativity

Fishman JA. Am J Transplant 2003
Burgos D. Transplant Proc 2006
Dharnidharka VR, et al. Transplantation 2009
Risk Factors for BK Infection

**Transplant risk factors:**
- Degree of overall immunosuppression
- Prior treatment for acute rejection-use of pulse steroids or lymphocyte-depleting agents
- Prolonged cold ischemia time and delayed graft function
- Ureteral stent placement (four-fold increase)
- Use of anti-thymocyte globulin induction, and tacrolimus (**TAC levels >10 ng/mL at month 3**) and/or MMF-based maintenance (**high MPA levels**)
- Renal injury (i.e. immune injury, proinflammatory cytokines, ischemia-reperfusion injury)
BK Virus-Donors

- Pretransplant samples from donor and recipients
- 54/81 adult kidney donors (67%): seropositive for BK virus
- BKV infection was seen in:
  - 46% recipients who received kidneys from seropositive donors
  - 15% recipients who received kidneys from seronegative donors
  - Suggesting direct correlation between the levels of BKV-specific antibody titer in the donor and transmissibility of BK infection

BK Virus-Donors

- **BK viruria** in recipients from seropositive donors occurred **earlier** (median onset: 45 days vs. 370 days, \( p < 0.001 \)) with **longer durations** (median duration: 157 vs. 7 days, \( p = 0.009 \)) and **higher peak urine titers**
- **Donor seropositivity**: associated with a significantly elevated odds ratio of 3.1
- Early BKV infection in kidney transplant recipients is usually of donor origin
- Testing for BK virus serostatus is neither routine, not mandated at this time

Recent prospective single center study; 192 adult recipients and 11 pediatric recipients

D+R- group had the highest incidence of BK infection
  - Onset was also much sooner in this group

The prevalence of BK infection, BK viremia, and significant viremia was lowest in the D-R- group

Similar results in pediatric population

Sood P, et al. Transplantation 2013
Donor Origin of BK Virus Replication

- Urine of 249 donor/recipient pairs tested for the presence of BKV-DNA by qPCR pre-and posttransplant.
- In 20 pairs, sequencing of the BKV VP1 typing region succeeded in donors and corresponding recipients posttransplant; the derived sequences were completely identical in donor and post-Tx recipient samples.

Polyomavirus BK Replication in *De Novo* Kidney Transplant Patients Receiving Tacrolimus or Cyclosporine

- **DIRECT** (Diabetes Incidence after Renal Transplantation: Cyclo vs Tac) study: a prospective 6-month, open-label multicenter study; 629 patients with a follow-up visit at month 12; between 2003-2005

- Tac dosing was based on C0 targets: 10–15 ng/mL during months 1–3 and 5–10 ng/mL during months 4–6

Polyomavirus BK Replication in *De Novo* Kidney Transplant Patients Receiving Tacrolimus or Cyclosporine

- Univariate analysis: CsA-MPA with lower rates of viremia than Tac-MPA at month 6 (10.6% vs. 16.3%, \( p = 0.048 \)) and 12 (4.8% vs. 12.1%, \( p = 0.004 \)) and lower plasma BKV loads at month 12 (3.9 vs. 5.1 \( \log_{10} \) copies/mL; \( p = 0.028 \))
- Multivariate models: CsA-MPA with less viremia than Tac-MPA at month 6 and month 12
- Viremia at month 6 was independently associated with higher steroid exposure until month 3, and with male gender and recipient age at month 12

Diagnostic Testing

- **Urine:**
  - Decoy Cells
  - DNA PCR
  - EM-Haufen
  - Urinary cytokines: IL-3 and IL-6 *(Sadeghi M, et al, 2009)*
  - Urinary cell mRNA profiles *(Dadhania D, et al. 2013)*

- **Blood:**
  - DNA PCR (Quant); PPV ~60%
  - Plasma BK level >10,000 copies/mL-associated with 93% specificity for presence of BK nephropathy *(Hirsch HH, et al. 2006)*

- **Renal biopsy:** Gold standard
Urine “Decoy cells”
(intranuclear viral inclusions in tubular epithelial cells)

• May be mistaken for degenerative or tumor cells
Haufen: Cast-like, Three-dimensional Polyomavirus Aggregates in the Urine

- Negative-staining electron microscopy: 194 urine samples from 139 controls (negative) and 143 samples from 21 patients (all positive) with BK nephropathy.

- Detection of Haufen (cluster or stack in German) in the urine correlated tightly with biopsy confirmed BK (concordance rate 99%).

Singh HK, et al. JASN 2009
Haufen: Cast-like, Three-dimensional Polyomavirus Aggregates in the Urine

- Densely arranged viral aggregates
- Haufen-positive urine sample: multiple small and large polyoma virus aggregates with significant Tamm-Horsfall protein content
- Comes from the injured renal tubular segments/out of the affected nephrons
- 100% sensitive and 99% specific for identifying biopsy-proven BK nephropathy
- Biomarker for intrarenal BK virus infection; noninvasive; not recommended as a mass screening tool at this time

Singh HK, et al. JASN 2009
Quantitative Urinary Polyomavirus-Haufen Testing

- Polyomavirus-Haufen were counted in 40 urine samples from patients with biopsy-proven definitive PVN.
- Polyomavirus-Haufen counts showed excellent correlation ($\alpha 0.77–0.86$) with the severity of intrarenal PV replication and disease grades.
- No correlations were seen with urinary decoy cell counts.

Singh HK, et al. Transplantation 2015
## BKV Screening Methods

<table>
<thead>
<tr>
<th>Screening method</th>
<th>PPV</th>
<th>NPV</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decoy cells</td>
<td>29%</td>
<td>100%</td>
<td>25%</td>
<td>84%</td>
</tr>
<tr>
<td>Haufen</td>
<td>97%</td>
<td>100%</td>
<td>100%</td>
<td>99%</td>
</tr>
<tr>
<td>BK urine PCR</td>
<td>40%</td>
<td>100%</td>
<td>100%</td>
<td>78%</td>
</tr>
<tr>
<td>BK blood PCR</td>
<td>50-60%</td>
<td>100%</td>
<td>100%</td>
<td>88%</td>
</tr>
</tbody>
</table>

Persistent BK viral load $>4 \log_{10}$ cp/mL 93% specific and 93% sensitive for biopsy-proven BK nephropathy

BK Virus Testing

- Viremia detection by molecular PCR amplification is associated with higher PPV and NPV for BK nephropathy than viruria by decoy cells or PCR.
- **Same laboratory and same assay - different primers and assay techniques**
- A negative biopsy does NOT rule out BK nephropathy due to the possibility of sampling error and the focal nature of the infection (sensitivity is not 100%).
- Biopsy: viremia is accompanied by elevation in serum creatinine or high-level viremia despite reduction of immunosuppression.
Penn DATA

- Since January 2008, kidney transplant patients have been prospectively screened by quantitative BK blood PCR (at 3, 6, and 12 months then yearly).
- Detectable BK viremia was defined as ≥2.6 log copies/mL.
- Retrospective analysis was performed for any patient transplanted between January 2008-December 2011 who had ≥1 BK viral load drawn until June 2012 at a protocol time-point.

*Trofe J et al, Abstract (Poster) ATC 2013*
### Time of First BKV Detection ≥ 2.6 log copies/mL at Protocol Time-Point

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>Count (Percentage)</th>
</tr>
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<tbody>
<tr>
<td>3 months</td>
<td>51 (48%)</td>
</tr>
<tr>
<td>6 months</td>
<td>34 (32%)</td>
</tr>
<tr>
<td>12 months</td>
<td>18 (17%)</td>
</tr>
<tr>
<td>24 months</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>36 months</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>48 months</td>
<td>None</td>
</tr>
</tbody>
</table>

- New onset BK viremia at ≥ 24 months was rare, questioning the utility of protocol screening beyond 24 months unless renal dysfunction is present.

*Trofe J et al, Abstract (Poster) ATC 2013*
Persistent BK Viremia-De Novo Donor Specific Antibodies (DSA)

- BK and DSA screening at 1, 3, 6 and 12 months; then yearly at Penn
- 785 patients; data collected 1/2008-12/2012
- 132 (17%) had detectable BKV during the study period
- The median time to BKV detection: 138 days (IQR 94-216); 48% of patients were diagnosed at 3-month routine screening time point
- Persistent BKV defined as lasting ≥ 140 days; 52% of the infected patients had persistent BKV

Sawinski D, et al. JASN 2015
Persistent BK Viremia - DSA

- After a median follow-up of 3 years, there was no significant difference in terms of patient or allograft survival between patients with and without BK viremia.

- Persistent BK viremia was strongly associated with the development of class II DSA but not class I DSA.

Sawinski D, et al. JASN 2015
### Persistent BK Viremia-DSA

Univariable and Multivariable Logistic Regression for Development of DSA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>P Value</td>
</tr>
<tr>
<td>Persistent BK viremia</td>
<td>2.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV</td>
<td>1.98</td>
<td>0.10</td>
</tr>
<tr>
<td>Donor age (yr)</td>
<td>0.99</td>
<td>0.05</td>
</tr>
<tr>
<td>PRA≥30</td>
<td>2.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>3.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Class I DSA</td>
<td>6.84</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Sawinski D, et al. JASN 2015
Persistent BK Viremia-DSA

- Consistent with prior studies, most BKV occurred within the first 3 months posttransplant
- Although immunosuppression reduction is an effective way to clear the virus from the blood, it is usually a slow and gradual process
- Compared with uninfected patients, neither the presence nor the duration of BKV had a deleterious effect on patient or graft survival
- Persistent BKV was associated with an increased risk of developing de novo DSA
Screening and Diagnostic Testing for BK

• AST Infectious Disease Community of Practice guidelines and KDIGO guidelines:
  • “Screening should be performed at least every 3 months during the first 2 years posttransplant, and then annually until the fifth year posttransplant”
  • “Earlier (starting at 1 month posttransplant) and more frequent screening (monthly plasma screening for the first 6 months, then every 3 months until 2 years posttransplant)”
    • May be more appropriate in high incidence transplant centers

• “In patients with sustained plasma BKV DNA and loads of >4 log\textsubscript{10} cp/mL, a diagnosis of ‘presumptive PyVAN’ should be made in absence of demonstrable BKV replication in biopsies”  
  

• Study in 413 patients:
  
  • A cutoff for plasma BKV of ≥4 log\textsubscript{10}/mL: the sensitivity of the commercial PCR Assay was very low (64.5%), but had a high specificity of 98.4%
  
  • 248 patients had renal biopsy, 31 (12.5%) had BKVN; 11 of the 31 (35%) had BKV consistently less than 4 log\textsubscript{10}/mL; current cut-off of ≥4 log\textsubscript{10}/mL underestimated the diagnosis of BKVN

  Hassan S, et al. Transplt Infect Dis 2013
Screening and Diagnosis

- Urgent need for:
  - Standardization of the various BK assays: some BK assays have decreased sensitivity for detecting less common BKV subtypes
  - Establishment of universal cutoff points
- Centers to develop their own assays and cutoffs until standardization
- Assays to assess BK-directed cellular immunity and anti-BK T cell phenotype monitoring responses to therapy
Treatment of BK Nephropathy

- Cidofovir, foscarnet, interferon, leflunomide, FK778, quinolones, and IVIG: no success
  - The addition of leflunomide or cidofovir to immunosuppression reduction does not result in a decrease in the rate of allograft failure
  
  Johnston O, et al. Transplantation 2010

- No specific antiviral drug treatment
- Careful reduction of immunosuppression and close follow-up for development of acute rejection (8-12%) - remains the cornerstone
- BKV-specific T cell transfer - needs further study
Treatment of BK Nephropathy

• Double-blind, placebo-controlled randomized trial in 154 patients who received a living or DD kidney-only transplant in 7 Canadian transplant centers between December 2011 and June 2013

• Randomly assigned to receive a 3-month course of levofloxacin (500 mg/d; n = 76) or placebo (n = 78) starting within 5 days after transplantation

• **Levofloxacin did not prevent BK viruria**; was associated with an increased risk of adverse events such as bacterial resistance

Knoll GA, et al. JAMA 2014
Clearing BKV Replication

- ELISpot assay was used: patients with TAC trough levels less than 6 ng/mL had significantly higher BKV large T-antigen-specific activity; TAC trough 3 ng/mL and CsA trough 100 ng/mL—even better results.
- Sirolimus, MMF, or leflunomide did not show a significant inhibition of BKV-specific-T-cell activation.

Treatment of BK Nephropathy

- TAC trough levels: 3 ng/mL, CsA trough levels 100 ng/mL, sirolimus trough levels <6 ng/mL, and MMF daily dose ≤1000 mg
- Switching from TAC to CsA, or from CNI to low-dose sirolimus, or from MPA to leflunomide or low-dose sirolimus-no randomized controlled studies

Treatment of BK Nephropathy

- Factors influencing viral clearing and renal function during BKVN:
  - High peak viral load
  - Tacrolimus treatment
  - Delayed diagnosis (biopsy for cause vs protocol biopsy)
  - Slow viral reduction time-switch from CNI to mTOR inhibitor favored viral load decrease

Treatment of BK Nephropathy

Many Questions Remain

- Multicenter prospective studies with longer follow-up and large-scale observational studies are needed:
  - Use of viral load or other techniques for a timely diagnosis
  - Modification of immunosuppression—what level of BK viral load to use and when?
  - Evaluation of different treatment strategies: what sequence of dose reduction+/-switching of agents?; optimal drug combination
  - Assessing the possibility of chronic allograft dysfunction and development of DSA due to systematic reduction of immunosuppression
Ongoing Studies

- Safety and Efficacy of Mycophenolic Acid Withdrawal With Conversion to Zortress (Everolimus) in Renal Transplant Recipients With BK Virus Infection: randomized, open-label-UCSF
- Ciprofloxacin use (500 mg daily for 3 months- to prevent BK infection-placebo-controlled-The Methodist Hospital System in Houston
- Using mTOR Inhibitors in the Prevention of BK Nephropathy: randomized, open-label, low dose TAC and MPA vs rapamycin and MPA: Columbia University-not sure if still ongoing
- Open-label trial of leflunomide and orotic acid use in patients BK viruria-no viremia no BKVN-multicenter trial-recruiting
- Haufen Diagnostic Biomarkers of BK Renal Disease-UNC, Chapel Hill and Astellas
- Use of Brincidofovir-lipid conjugate of cidofovir; increased antiviral potency against dsDNA viruses-to be seen
Re-Transplantation

- Possible despite persistent/increased risks
  - The short-term graft and patient survival with re-transplantation after BK appears to be excellent
  - Longer-term outcomes remain unknown
    

- Not enough data to recommend nephrectomy of failed allograft prior to re-transplantation
  - **Nephrectomy is not necessarily protective**

- Pre-transplant clearance of viremia is necessary
Follow-up

- Reduction of immunosuppression: MPA was stopped; tacrolimus dose was reduced to a target level of 3-4
- BK viral load continued to be high: switched to cyclosporine—currently on cyclosporine (last level 40) and prednisone (5 mg daily)
- Last BK viral load in August 2015 (over 5 years posttransplant): 2.6 log copies/mL and scr 1.8 mg/dL; DSA negative
Summary

- Outcome of established BK Nephropathy = NOT VERY GOOD
- No direct immunoprophylactic strategy/drug
- No vaccine against BK virus
- Screening is very important
  - Blood BK viral load
- Immunosuppression reduction: patients should be closely monitored with a serum creatinine checked every 1-2 weeks and BK viral load repeated at 2-4 week intervals