Renal Histo-Immunopathology at square two:
Polyoma-BK-virus Nephropathy and Acute Rejection

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Case History Biopsy #1:

The patient is an 18 year old male who had undergone renal transplantation 6 months ago.

- Underlying native kidney disease: ANCA mediated necrotizing and crescentic glomerulonephritis
- Transplanted kidney received from mother, one haplotype match.
Case History Biopsy #1:

- Baseline immunosuppression: Tacrolimus, Mycophenolate Mofetil, and Prednisone.
- The patients presented with an elevation of the baseline serum creatinine from 1.0 to 1.7 mg/dL.

A renal biopsy was performed.
Low power view:
- 45 glomeruli total within normal limits.
- Most of the tubulointerstitial compartment was unremarkable.

Medium power view:
- Small aggregates of mononuclear cell in the interstitium associated with rare foci of minimal tubulitis, edema and focal intratubular calcium deposits.
Biopsy #1:

- C4d was not detected along peritubular capillaries.
- Arterioles and arteries were within normal limits.
- There was no evidence of transplant endarteritis.
Immunostain for SV40 Large T Antigen: Very rare (circled) tubular epithelial cells demonstrated intranuclear positivity.
Biopsy #1:

- What is the diagnosis?
- What other laboratory test results would be helpful to further support your diagnosis?
- How should the patient be managed?
Diagnosis – Biopsy #1:

• Polyoma BK virus Nephropathy (early stage A) associated with focal minimal interstitial inflammation and focal tubular calcifications.

• No evidence of acute rejection.
Biopsy #1:

• What is the diagnosis?

• What other laboratory test results would be helpful to further support your diagnosis?

• How should the patient be managed?
• Urine cytology for decoy cell quantification.
• Plasma PCR for BK virus load levels
• Urine PCR for BK virus load levels


Biopsy #1:

• What is the diagnosis?

• What other laboratory test results would be helpful to further support your diagnosis?

• How should the patient be managed?
Clinical Course:

• The patient’s immunosuppressive regimen remained unchanged and no other treatment was instituted.

• The patient presented two months later with an elevation of the serum creatinine up to 2.0 mg/dL.

• A second renal biopsy was performed.
Of Note:

Based on the positive immunostaining for SV40 Large T antigen indicating a productive polyomavirus infection (virus in a replicative state), and in the absence of acute rejection, the patient’s immunosuppression should have been reduced and an antiviral agent such as leflunomide or cidofivir should have been added.
Biopsy #2: Low power view of inflammatory infiltrate concentrated in the medulla (area in box) with a minor component in the cortex.
Biopsy #2: Mostly in the medulla, typical intranuclear viral inclusions were seen (circled). Associated cell lysis was seen within involved tubules (arrow).
Biopsy #2: Tangential section through arterial wall with subendothelial CD3 (T cell) positive mononuclear cells: transplant endarteritis.
Biopsy #2:

• Complement degradation product C4d was not detected along peritubular capillaries.

• Incubations to detect Epstein-Barr virus were unrevealing.

• Immunofluorescence incubations did not reveal any evidence of glomerular immune deposits.
Biopsy #2:

• What is your diagnosis?

• What do you do with the patient now?
Diagnoses – Biopsy #2

• Polyoma-BK-virus Nephropathy (BKN) – Stage B1.

• Cellular allograft rejection involving tubulointerstitial compartment and arteries (transplant endarteritis), Banff 2A cellular rejection (C4d negative).

Biopsy #2:

- What is your diagnosis?
- What do you do with the patient now?
Two Step Treatment Strategy in cases with **combined** BKN and acute rejection:

- Treat the acute rejection first, then, lower immunosuppression for BKN.

- Addition of an antiviral agent – i.e. Cidofovir, Leflunomide. +/- Stop MMF.

- Monitor patient serially with urine cytology for decoy cell quantification and plasma PCR for BK virus load levels to follow disease course.
Polyomaviruses BK Nephropathy (BKN)

- Background
- Morphological Aspects & Diagnosis
- How to establish an early Diagnosis (Screening)
- How to manage patients
Polyomavirus BK Nephropathy (BKN)

• Background

• Morphological Aspects & Diagnosis

• How to establish an early Diagnosis (Screening)

• How to manage patients
Polyomaviruses

- Non-enveloped double stranded DNA viruses of 5000 – 5300 base pairs
- 40 - 45 nm in diameter
- Icosahedral symmetry with 72 pentameric capsomers
- VP1 capsid protein 70% of virion mass; antigenic properties
Polyomaviruses
- in humans -

- Three strains pathogenic:
  - BK-Virus*
  - JC-Virus*
  - (SV-40 Virus)

- High gene sequence homology
  (JC/BK: 75%, JC/SV40: 70%)

* first isolated from immunocompromised patients in 1971; named after patients’ initials
Polyomavirus

- Primary infections (JC, BK) common early in life (median age: 4-5 years)

- Seropositivity:
  90% - 100% in children at the age of 10 years
  46% - 94% in adults (77%*)

*NEJM 347 (7): 488, 2002
Polyomavirus

- After primary infection: latency
- **BK virus**: urotropic
  most common latency in the uro-genital tract
  (transitional cells, kidney)
- **JC virus**: neurotropic
  most common latency in B-cells, tonsils, kidney, ? brain
Polyomavirus

- Cell entry via endocytosis,
  - VP-1 (viral capsid) and receptor mediated.

- Viral replication in host cell nucleus with host replicative enzymes.

- Viral release via host cell necrosis and lysis → free viral particles
Cell entry via endocytosis, VP-1 (viral capsid) and receptor mediated.
Polyomavirus: Tubular cell cytoplasm
Polyomavirus virions in **cell nucleus** – replication using host replicative enzymes
<table>
<thead>
<tr>
<th>POLYOMAVIRUS INFECTIONS: TERMINOLOGY</th>
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</thead>
<tbody>
<tr>
<td><strong>Viral Activation</strong></td>
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<tr>
<td>Evidence of polyomavirus replication: (1) decoy cells or free virions in urine; (2) viral detection by PCR in urine, serum, CSF fluid. Often seen as a transient and asymptomatic event. - ALSO - is part of viral disease.</td>
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<tr>
<td><strong>Viral Disease</strong></td>
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<tr>
<td><em>BK polyomavirus Nephropathy;</em></td>
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<td><em>Hemorrhagic cystitis after BM-Transplant;</em></td>
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<tr>
<td><em>Progressive Multifocal Leukoencephalopathy</em></td>
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<tr>
<td>Histologic evidence of viral activation in <strong>organs</strong> (cytopathic changes and/or positive IHC or in-situ hybridization) &amp; associated virally induced tissue injury (i.e. kidneys, bladder, brain), often with clinical symptomatology.</td>
</tr>
</tbody>
</table>

All patients with BKN show signs of “viral activation”

BUT

not all patients with signs of viral activation have manifest disease, i.e. BKN.
Polyomavirus -BK- Nephropathy (BKN)

- Mid-1990’s:
  - New immunosuppressive drugs (Tacrolimus; Mycophenolate-Mofetil) introduced into routine use in renal transplant patients worldwide.
    - (> 90% of patients in the western world)

- BKN became a major complication in renal allograft recipients.
BKN

- Most common infectious complication affecting kidney transplants.

- Incidence: 1% - 10% worldwide
  - 6.5% incidence at The University of North Carolina at Chapel Hill, USA.

- Graft failure rates, especially when diagnosed late, can reach 50 – 80% within 24 months.
BKN

• Manifestation 12 mths post transplantation (range: 6 days to 6 years)

• Limited to renal allograft (one exception: NEJM 345 (17): 1250-1255, 2001)

• Exceptional cases of native kidney infection after heart, pancreas, bone marrow transplantation or with lymphomas.
BKN

- Currently, a **definitive** diagnosis of BKN made only by renal biopsy and detection of characteristic histological changes.

- **Aggressive screening** is recommended to identify patients at risk for BKN to:

  - **optimize timing** for renal biopsy in order to detect early stage disease.
BKN

• Presentation: **No characteristic findings, can see:**
  Serum creatinine **normal** - elevated
  No hematuria
  No proteinuria
  No generalized symptoms, e.g. fever

  **Caveat: BKN can even be found in protocol biopsies !**

• **ALL patients show signs of viral activation in urine and plasma**
Polyomavirus BK Nephropathy (BKN)

- Background
- Morphological Aspects & Diagnosis
- How to establish an early Diagnosis (Screening)
- How to manage patients
• Diagnosis **best** by histology
• Virally induced changes in epithelial cells are the characteristic defining feature: tubular >> parietal epithelial cells
Polyoma BK-Virus Nephropathy

Viral replication in tubular epithelial cells

- Intranuclear viral inclusion bodies
- Ultimately leading to cell lysis and
- Denudation of tubular basement membranes

Acute tubular epithelial cell necrosis
Characteristic viral inclusion bearing epithelial cells with associated cell lysis and denudation of tubular basement membranes – BKN florid disease stage (Stage B).
SV40 T antigen expression in tubular nuclei
Virally induced ATN

Focal → no / mild impact on function
Diffuse → severe impact on function
Early changes → complete recovery
Late changes → irreversible alterations
- interstitial fibrosis
- tubular atrophy
Polyomavirus Allograft Nephropathy: Disease Stages

(from ‘Heptinstall’s Pathology of the Kidney’, 6th edition, Table 28.16)

**Stage A**
(early changes)
- Viral activation in cortex and/or medulla with intranuclear inclusion bodies AND/OR positive immunohistochemical or in-situ hybridization signals
- No or minimal tubular epithelial cell necrosis/lysis
- No or minimal denudation of tubular basement membranes
- No or minimal interstitial inflammation in foci with viral activation
- No or minimal tubular atrophy and interstitial fibrosis ($\leq 10\%$)

**Stage B**
(florid changes)
- **Marked** viral activation in cortex and/or medulla
- **Marked** virally induced tubular epithelial cell lysis and associated denudation of tubular basement membranes
- Interstitial inflammation (mild to marked)
- Interstitial fibrosis and tubular atrophy
  
  *Stage B1* - $\leq 25\%$ of specimen involved
  *Stage B2* - $> 25\%$ and $< 50\%$ of specimen involved
  *Stage B3* - $\geq 50\%$ of specimen involved (and $\leq 50\%$ fibrosis)

**Stage C**
(advanced sclerosing)
- Viral activation in cortex and medulla
- **Interstitial fibrosis and tubular atrophy $> 50\%$ of sample**
- Tubular epithelial cell lysis and basement membrane denudation (minimal to marked)
- Interstitial inflammation (minimal to marked)
Polyoma BK-Virus Nephropathy: Stage A

IHC: SV40 T antigen
Polyoma BK-Virus Nephropathy: Stage B

IHC: SV40 T antigen
Polyoma BK-Virus Nephropathy: Stage C
Interstitial Inflammation
Interstitial Inflammation

BKN

Caveat: Consider concurrent rejection in BKN stage A with “abundant” lymphocytic infiltrates

- lymphocytes (B- and T-cells)
- plasma cells
- polymorphonuclear leukocytes
- histiocytes

due to

- “virally” induced nephritis
- AND / OR
- concurrent acute rejection
BK-Virus Nephropathy

How to diagnose concurrent rejection:

**Immuno-histochemistry**

1) Tubular expression of HLA-DR (MHC Class II)
2) Accumulation of C4d along peritubular capillaries
Tubular HLA-DR

Typical cases of rejection

Capillary C4d
BK virus replication in tubular cells stimulates MHC class I but not MHC class II expression

V. Nickeleit et al., Nephrol Dial Transplant 15: 324-332, 2000

Immunofluorescence double incubation:
- MHC-class II (HLA-DR) expression green
- BK-virus antigens orange

BK virus nephropathy is not associated with the deposition of C4d along peritubular capillaries

V. Nickeleit et al. JASN 13: 242-251, 2002
Agenda: Polyomavirus BK Nephropathy

- Background
- Morphological Aspects & Diagnosis
- How to establish an early Diagnosis (Screening)
- How to manage patients
Adjunct clinical parameters for patient screening

Currently use:

A) PCR on plasma and urine for BK virus load levels.

B) Decoy Cell quantification in urine cytology.
PCR Tests and Decoy Analyses:

All patients with BKN show signs of “viral activation”

BUT

not all patients with signs of viral activation have manifest disease
Decoy-Cells*

- Viral inclusion bearing cells
- Typically contain polyomavirus virions
- May be mistaken for degenerative or tumor cells
- Mark viral (re)activation

*name coined by L.G. Koss and colleagues
Decoy-Cells

- Papanicolaou stained preparations:
  - Smears, cytospins, monolayer “thin prep”

- Quantification:
  - significant: > 10 decoys / ThinPrep slide or cytospin.
  - > 5 decoys/10hpf in smears.
Decoy Cells in Urine

Asymptomatic, no viral disease
All samples: 0.5 %
Pregnant women: 3 %
Diabetic patients: 3 %
Cancer Patients: 13 %
Healthy kidney transplant recipients: 23 %

Symptomatic, viral disease
Patients with BKN: >95 %
Quantitative PCR analyses:
Threshold levels to mark “presumptive” BKN

<table>
<thead>
<tr>
<th>URINE</th>
<th>Plasma</th>
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<tr>
<td>DNA*</td>
<td>VP1 mRNA**</td>
</tr>
<tr>
<td>$1 \times 10^7$</td>
<td>$6.5 \times 10^5$</td>
</tr>
</tbody>
</table>

* J Clin Microbiol 42 (3):1176, 2004
** Transplantation 74: 987, 2002
*** NEJM (347) 7: 488, 2002

Caveat: Cut-off points are undetermined!!  
Cut-off points are only significant at time of diagnosis!!
Quantitative PCR analyses for BK-virus DNA / RNA loads with readings exceeding “threshold levels”:

Improved “positive predictive values”
~ 80%: presumptive PVN
Urine Electron Microscopy:

Search for three dimensional polyomavirus aggregates ("Haufen") as specific markers of Intra-tubular viral replication / BKN
Agenda:
Polyomavirus BK Nephropathy

• Background

• Morphological Aspects & Diagnosis

• How to establish an early Diagnosis (Screening)

• How to manage patients
Patient management

Problems

• Early diagnosis, stage A ("Screening")

• Detection of viral clearance during treatment ("Monitoring")

Aim

• Healing of ATN without atrophy / scarring
Patient screening period

1) Urine cytology
   - Negative (no risk)
   - POSITIVE (>10 decoy cells per ‘ThinPrep’ specimen)

   Low Risk: Level 1

2) PCR on plasma or urine to detect BK-Virus
   - Negative (no/minimal risk)
   - POSITIVE (>1 × 10^4 BK copies/ml plasma*)
   - (>1 × 10^7 BK copies/ml urine*)

   High Risk: Level 2

Diagnosis: Renal biopsy

- Kidney biopsy
  - No BKN
  - BK virus nephropathy (BKN)
    (high risk: level 2, close surveillance)

  - Rejection present
  - Rejection absent
    - Transient anti rejection therapy

Persistent BKN – Patient treatment and monitoring period

- Therapy attempts (for many weeks to months)
  - (e.g. low-dose immunosuppression, cidofovir, leflunomide)

- Decoy cells
- PCR on plasma

- Positivity (even low levels*)
- Persistent BKN
  - Results turn from positive to negative
  - Results remain negative for weeks

- DECOY CELLS, PCR: BOTH NEGATIVE

Resolution period – BKN is overcome

- Kidney biopsy for confirmation optional

* Significant PCR threshold levels are not definitively established; plasma load analyses are best suited for patient management.

Rejection and BKN can coincide


*Am. J. Kidney Dis.* (38) 3: E13, 2001

*NEJM* (347) 7: 488, 2002

*GRAFT* (5, supplement December): S46, 2002


*Polyomavirus Consensus Conference, Basel* 2003

*(Transplantation 2005, in press)*
BK virus nephropathy and concurrent rejection can be successfully treated in a two step approach
Transient anti-rejection therapy does not result in “explosive” BK virus replication.
AJKD 38 (3): E13, 2001

S-Cr

BK virus DNA in plasma, absent: △ present: ▲

Decoy cells in urine, absent: ○ present: ●
Polyomavirus BK Nephropathy:
clinico-pathologic correlations and the pathologist’s role in patient management
(Cytopathologists, Histopathologists)

...crucial for
- Risk Assessment
- (non-invasive) Diagnosis of BKN
- Disease Staging
- Disease Monitoring