DECOY CELLS
IN KIDNEY TRANSPLANT RECIPIENTS

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The finding of decoy cells in the urine indicates the reactivation of a polyomavirus (PV) within some epithelial cells of the urinary tract. In most cases, this PV is represented by BK virus (BKV).

What are PVs and what is a BKV?
POLYOMAVIRUS FAMILY & CLINICAL CORRELATES

- **BK VIRUS**
  - Kidney transplant recipients:
    - BKV nephropathy (and ureteral stenosis)

- **JC VIRUS**
  - Bone marrow transplant recipients:
    - Hemorrhagic cystitis

- **KI VIRUS** (Karolinska Institute)*

- **WU VIRUS** (Washington University)*

*Identified by screening human respiratory secretions
Their role in respiratory diseases still unclear

Share

SV40
large T antigen
PV FEATURES

- Double-stranded DNA
- Naked
- Icosahedral
- 40-44 nm in diameter
- Composed of 5,000 bases
- With a genome encoding 6 known proteins: 3 capsid + 3 noncapsid
- With also a nonencoding area
EPIDEMIOLOGY

• **Primary infection:**
  Early childhood through upper respiratory tract or gastro-enteric tract

• **Route of infection:**
  Non oral (air, semen, blood products, placenta, organ transplantation)
  Oral (contaminated food or water)

• **Latency phase within:**
  Kidney   Brain
  Ureter   Lymphoid cells

• **Diffusion:**
  80-90% of the general population
CONDITIONS ASSOCIATED WITH REINFECTION

- Pregnancy
- Diabetes mellitus
- HIV infection
- Cancer
- Kidney and other organ allografts
BKV REACTIVATION IN KIDNEY TRANSPLANT RECIPIENTS
TIME OF REACTIVATION

• Reactivation of BKV is common during the 1st post-transplant year, with a prevalence of 40-50%

• BKV nephropathy is much less common and occurs mostly in the first 2 years after transplant (only 5% of cases being observed between the 2nd and the 5th year)
MAIN FACTORS INFLUENCING THE REACTIVATION OF BKV

The intensity of immunosuppression rather than a specific drug and

Synergizing factors:

Patient features
(age >50 yrs, male gender, BKV seronegative recipient)

Graft features
(BKV sero+ donor, HLA mismatches, ischemic or immune injury)

Virus features
(latent viral load, capsid serotype, replicative fitness)
DIAGNOSIS OF BKV REACTIVATION

KDIGO clinical practice guideline for the care of kidney transplant recipients

Am J Transplant 2009; 9 (suppl 3): S1-S157
KDIGO GUIDELINE: THREE DIAGNOSTIC TOOLS

• Nucleic acid testing *(plasma)*
• Nucleic acid testing *(urine)*
• Decoy cells in urine

Nucleic acid testing = NAT
The use of NAT in plasma provides a sensitive method for identifying BKV infection and determining patients who are at increased risk for BKV nephropathy (BKVN).

Although plasma NAT assays lack standardization, a threshold plasma BKV level of >10,000 copies/mL is associated with a 93% specificity for the presence of BKVN.
NUCLEIC ACID TESTING: URINE

“A negative urine NAT has almost a 100% NPV... However, the presence of a positive NAT in urine in the absence of an elevated BKV load in plasma is NOT associated with an increased risk for BKVN. Hence, a positive NAT in urine requires performance on the blood. This requires patients to return to the clinic for the additional test.”

“Accordingly, it is suggested that NAT be performed on plasma and NOT on urine”
"When NAT is not available, microscopic evaluation of the urine for the presence of DCs is an acceptable, albeit nonspecific, alternative method...

A negative test rules out BKVN in most cases (=high NPV)...However, a positive test has a very low PPV for BKVN

It may be inappropriate to change therapy in such patients based on the presence of DCs alone"
KDIGO RECOMMANDATION FOR THE SCREENING OF BKV

• Use quantitative plasma at least:
  Monthly for the first 3–6 mos
Then, every 3 mos until the end of the 1st year
and
  • Whenever there is:
    An unexplained rise S-creat
After treatment for acute rejection
HOWEVER,
SOME AUTHORS HAVE A
DIFFERENT VIEW
Intensive decoy cells (DC) surveillance program:

• TX to month 2: DC fortnightly
• Month 3 to 6: DC monthly
• Month 7 to 12: DC every 2 months
RESULTS

313 patients (KT 211 + SKPT 102)

- Sustained DC positivity*: 32 (KT: 24; SKPT: 8)
- Viremia: 24 (KT: 18; SKPT: 6)
- BKV nephropathy: 3
- Median time (days) for positivity:
  Sustained DCs: 67 days; viremia: 105 days
- BKVN developed only in patients with DC & viremia

*= ≥2 positive samples >2 weeks apart
“The practice of the Oxford Transplant Unit is to use DC assessment for the initial BKV surveillance, with only those patients identified as having sustained DC positivity progressing to NAT on blood...

With this approach, on the patient population described over a two-year period, there was a net saving of £135,000 when compared with routine PCR surveillance”
DETECTION OF VIREMIA
(Chakera A et al. Transplantation 2011; 93: 1018-23)

- High sensitivity
- Variable accuracy depending on the primers used & BKV serotypes present in the population
- Expensive (~ £ 86/sample)
- Not always available in house, with potential delays in obtaining results and limitations in clinical utility
DETECTION OF DECOY CELLS
(Chakera A et al. Transplantation 2011; 93: 1018-23)

• Low sensitivity
• High specificity and high NPV
• Cheap (~ £ 1/sample)
• Requires a trained cytopathologist
• Can be influenced by the timing of urine sampling and delays in processing
• Can be available within 60 min if required
212 kidney transplant recipients, prospectively investigated by the serial evaluation of decoy cells in the urine.

Results and conclusions similar to those reported by Chakera et al.
DECOY CELLS

Singh HK, Bubendorf L, Mihatsch MJ, Drachemberg CB, Nickeleit V.

*Urine cytology findings of polyomavirus infections.*

Adv Exp Med Biol 2006; 577: 201-12
ORIGIN

- The origin of DCs cannot easily be discerned based on morphologic ground.
- In healthy patients, they would likely originate from the urothelium, especially from its superficial layers.
- In case of BKVN, DCs likely originate from the renal parenchyma, probably through the ascending route of infection, from urothelium to collecting ducts and proximal tubular cells.
MORPHOLOGY

• **FOUR DIFFERENT PHENOTYPES** which depend upon the state of viral replication and maturation as well as the state of cellular preservation and

• **HYBRID FORMS** which represent transitions between the different phenotypes and are frequently found in the same specimen
THE FOUR PHENOTYPES

1. Ground glass or gelatinous nuclear appearance

2. Intranuclear inclusion surrounded by a clear halo, i.e., CMV-like

3. Multinucleated cells

4. Vesicular nuclei with clumped chromatin and nucleoli

plus

Eccentric nuclei and dropletlike appearance
“DCs can best be detected and quantified in standard alcohol fixed and Papanicolaou stained urine cytology specimens from either smeared or cytocentrifuged (i.e. cytospin) urine samples”

“...much is still dependent on the experience level of the pathologist”
Homogeneous, amorphous ground-glass like nucleus appearance with intranuclear inclusion bodies (arrows)
CMV-like DCs with central intranuclear viral inclusion bodies surrounded by irregular halos (arrows). The cell on the left with a typical comet-like appearance.
DCs with granular chromatin pattern and multinucleation
DCs with vesicular nuclei and distinct network of coarsely granular and clumped chromatin
“DCs can also be detected in unstained urine sediment using phase contrast microscopy. This technique, however, requires great experience and the quantification of DCs is tricky”
**DCs DETECTED WITH PHASE CONTRAST MICROSCOPY**

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<thead>
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<th>AUTHOR</th>
<th>JOURNAL</th>
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<td>Binet I et al</td>
<td>Transplantation 1999; 67: 918-22</td>
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<tr>
<td>Huang G et al</td>
<td>Diagn Microbiol Infect Dis 2013; 75: 292-7</td>
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Images in Nephrology
(Section Editor: G. H. Neild)

‘Decoy cells’ in the urine due to polyomavirus BK infection: easily seen by phase-contrast microscopy

Giovanni B. Fogazzi, Mariadele Cantú and Lucia Saglimbeni

Divisione di Nefrologia e Dialisi, Ospedale Maggiore, IRCCS, Milano, Italy
TYPE 2 PHENOTYPE
TYPE 4 PHENOTYPE
COMET-LIKE CELLS
HYBRID FORMS
DECOY CELL CASTS
DECOY CELL CASTS
WHY PHASE CONTRAST?

• It allows an easy identification of the morphological changes caused by BKV

• It is based on the same procedures and equipment used in everyday work (= simple, fast and inexpensive)

• It does not require cytologic techniques (stain, smear, cytospin), which are available only in specialised laboratories
STANDARDISED METHOD FOR THE DETECTION OF DCs BY PHASE CONTRAST IN OUR LABORATORY

• 10 mL of the 2nd urine of the morning produced over 2 hours
• Centrifugation at 400 g (2,000 rpm) for 10 min
• Removal of 9.5 mL of supernatant urine
• Resuspension of the sediment in the remaining 0.5 mL of urine
• Transfer to a glass slide of 50 μL of the resuspended sediment, which is then covered with a 24 x 32 mm coverslip
• Counting of DCs over 50 HPFs (400x)
• Result expressed as number of DCs/50 HPFs
PAPANICOLAOU STAIN: OUR EXPERIENCE
(43 samples from 18 patients)

• Same morphological changes as found with phase contrast

• However, Papanicolaou stain associated with:
  - Some better details of nuclear inclusions
  - Inconsistent quality of the stain
  - More complex & longer procedures than phase contrast (40 vs 15 min)
Type 4

Type 1
ELECTRON MICROSCOPY
TYPICAL FINDINGS*

• Viral particles with a diameter of about 40-50 nm
• Occasionally, viral particles are aggregated in crystalloid arrays
• In rare cases, DCs contain adenovirus particles, which by EM have a diameter of about 80 nm

*Singh et al. Urine cytology findings of polyomavirus infections
Adv Exp Med Biol 2006; 577: 201-12
ELECTRON MICROSCOPY

Our experience on 11 samples from 10 patients*

- **AIM:** to investigate whether what we identified as DCs by phase contrast contained intranuclear viral particles

- **RESULTS:** typical intranuclear viral particles were found in the cells of 10/11 samples (the negative sample was not suitable for a proper investigation due to the very low number of cells)

* Kidney transplant recipients = 10; lymphoma = 1
Enlarged with viral particles & chromatin margination
Enlarged nucleus with viral particles & intranuclear inclusions
Nucleus packed viral particles
Comet-like cells with chromatin margination and clumped viral particles
DECOY CELLS:
POSSIBLE MISINTERPRETATIONS

• Malignant tumor cells (MTCs)
• Cytomegalovirus (CMV) cells
MALIGNANT TUMOR CELLS (1)

- MTCs: dark, coarse and randomly distributed with hyperchromatic material with prominent nucleoli and no homogeneous central part of the nucleus
- No eccentric nuclei
- Tendency to clustering with crowding or nuclear overlap
“However, the distinction of DCs from MTCs can be impossible on cytomorphology alone”

“Therefore, ancillary techniques are necessary to differentiate DCs from MTCs” (e.g., EM of the urine, FISH profile)

Galed-Placed I & Valbuena-Ruvira L.
Decoy cells and malignant cells coexisting in the urine from a transplant recipient with BK virus nephropathy and bladder adenocarcinoma. Diagn Cytopathol 2011; 39: 933-7
OUR PRACTICAL APPROACH

In our view, BKV reactivation and urothelial cancer may be differentiated on the basis of global urine sediment findings.

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<tr>
<th>URINE PARTICLE</th>
<th>BKV INFECTION</th>
<th>UROTHELIAL CANCER</th>
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<tr>
<td>EPITHELIAL CELL CLUSTERS</td>
<td>Absent</td>
<td>Present</td>
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<tr>
<td>RBCs</td>
<td>Absent</td>
<td>Present</td>
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<td>WBCs</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>MACROPHAGES</td>
<td>Present</td>
<td>Absent</td>
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CYTOMEGALOVIRUS CELLS

• Large cells containing intranuclear viral inclusions bodies surrounded by a clear halo (“owl’s eye” appearance)

• Eosinophilic cytoplasmic viral inclusion bodies possible (by Papanicolaou)

• Ground glass appearance uncommon
Several studies from different groups have demonstrated that:

1. The finding of DCs in the urine does indicate a reactivation of BKV

2. However, the finding of DCs does not indicate per se the presence of BKVN, the PPV of DCs for this condition being only 27-29%
WHAT IS, THEN, THE UTILITY OF MONITORING DCs IN THE URINE?

1. The finding of DCs should alert the clinician about a possible evolution towards BKVN and, hence, prompt a close monitoring of the patient and action (i.e., reduction of immunosuppressive treatment)

2. The PPV of DCs for BKVN increases to >90% if:
   - The No of DCs is high*
   - The shedding of DCs persists over time
   - DC casts are also found
   - DCs is associated with the development of viremia & of renal dysfunction

* Positive correlation between the No of DCs and the severity of BKVN
FURTHER EVIDENCE OF THE UTILITY OF MONITORING DCs IN THE URINE

Close correlation between the persistence/clearance of DCs and the persistence/healing of BKVN at renal biopsy


Thank you for your kind attention