TRACING OF “DECOY CELLS”: RAPID SCREENING TEST FOR DEMONSTRATION OF ACTIVE POLYOMAVIRUS INFECTION IN PATIENTS WITH RENAL TRANSPLANTS AND UROLOGIC DISORDERS

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ABSTRACT
Reactivation of polyomavirus hominis 1 (BK virus, BKV) is an important cause of the morbidity in kidney transplant patients. This seemingly insignificant viral infection that affects the majority of population at a young age, once reactivated by immunosuppression, is a major factor contributing to graft loss. Screening techniques have been developed for early prediction of BK virus reactivation. These include plasma and urine assays for detection of BK virus DNA by PCR, urine cytology for detection of “Decoy cells” and electron microscopy. Once screening tests reveal a suspicious BK virus reactivation, tissue biopsy should be performed to confirm the diagnosis, rule out acute cellular rejection and plan treatment approaches. BK virus-associated nephropathy is an emerging cause of kidney transplant loss. In this study, 75 patients with different clinical diagnoses were subjected to urine cytology diagnosis. The group of patients with complications after renal transplantation shows much higher shedding of “Decoy cells” which is in correlation with their status and immunosuppressive therapy.

Keywords: BKV, decoy cells, renal transplantation

Introduction
The polyomavirus hominis 1 (BK virus, BKV) plays major role in the cytopathology of the urinary system. Primary infection with BK virus is acquired during the early childhood and is generally asymptomatic. After primary infection, the virus may establish life-long latency in many sites, most notably in transitional cell layer of the bladder, ureters and the renal pelvis as well as the tubular epithelial cells of the kidney (11). Renal tubular epithelial cells and their compensatory proliferation to restore tubular integrity after immunologic, ischemic or toxic injury may provide the critical cellular milieu supporting polyomavirus replication while immune control is impaired due to maintenance immunosuppression, anti-rejection, treatment and HLA- mismatches. Patients determinants (older age, male gender, seronegative recipient), and viral factors (genotype, serotype) may have a contributory role (4). Immunocompetent individuals rarely suffer from reactivation, but BK virus causes clinically manifest disease in immunocompromised hosts - bone marrow or kidney allografts as well as HIV infected patients. It is designated as BK polyomavirus associated nephropathy, (BKN, PVAN) or hemorrhagic cystitis in bone marrow patients (5).

In permissive infections, the BKV produces large homogenous basophilic nuclear inclusions that occupy nearly the entire volume of the enlarged nucleus. Rarely a narrow rim of clearing separates the inclusion from the nuclear envelope. The infected cells are often also enlarged. With the passage of time the inclusions become less basophilic and acquire homogenous appearance. Electron microscopic studies of the infected cells disclosed a crystalline network of viral particles, each measuring about 35 nm in diameter.

The use of urine cytology for diagnosis of BKV shedding has been documented since the 1970s (8). The abnormalities were first recognized in the 1950s by A. Ricci, who named these cells “Decoy cells” (7). Current methods have begun to evaluate noninvasive tests for the diagnosis of BK virus infection and disease. Cytopathologic assessments as well as electron microscopy of the urine sediments have a role in identifying BK virus disease. Evaluating of the urine for presence of “Decoy cells”, which is suggestive for viruria, offers a simple screening test of urine (1). Using this improved diagnostic tool as well as histopathology and plasma viral load measurement may lead to an early diagnosis of BKN before severe allograft dysfunction (3).

Materials and Methods
Urine samples – collection and treatment
Seventy-five urine samples were collected from patients at the University Hospital “Alexandrovskaya”, Medical University-Sofia. Ten urine samples from healthy individuals were studied in the same conditions and served as a control group. First or second voided urine was preferred and 50 ml containers (Biologix Res. Co., USA) supplied with 5 ml of ethanol, which prevents from bacterial growth and destruction of the urothelial...
cells were used (2). After collection they were transported using IsoTherm System (Eppendorf, Germany) to the laboratory and were immediately processed for the preparation of glass-slide smears.

Smears from all urine samples were prepared using the semiautomated Bales’ method (7). In brief, the preparation of the smears includes centrifugation at 1500 rpm, decanting the supernatant and vortex, adding from 3 to 5 ml 2% of Carbowax in 70% ethanol to the sediment, centrifugation at 1500 rpm, decanting the supernatant and using 50 μl of the sediment. Two glass slides of each sample were prepared. The samples were air-dried and then fixed for 10 min in absolute alcohol before applying the stain procedure.

Urine cytology
Papanicolaou stain (Cytocolor Cat.No.1.15355.0001; Merck KGaA) was performed according to manufacturer instructions (7). It included the following 10 steps: (a) Rinsing the glass slide under running water for 1 min; (b) Hematoxylin for 1 min; (c) Rinsing under running water till the hematoxyline is completely discarded; (d) Dipping in 2-propanol for 5 times; (e) Polychromic solution for 1 min; (f) 80% 2-propanol (5 times); (g) Absolute 2-propanol (5 times); (h) Absolute 2-propanol (5 times); (i) Xylene-1 (5 times); (j) Xylene-2 (5 times). The stained microscopic slides were immediately embedded into Canada balsam and observed for typical “Decoy cells” bearing viral inclusions by Carl Zeiss light microscope.

Results and Discussion
In thirty-three of all examined urine samples (44%) were discovered typical “Decoy cells” (Fig. 1).

Fig. 1. Photograph of “Decoy cell” from a patient with chronic renal failure. The enlarged nucleus with the viral inclusion body is easily recognized (x100).

The distribution of the obtained positive results according to the different diagnoses is presented in Table 1.

Obtained results demonstrate that the number of positive for “Decoy cells” urine samples is significantly higher in the patient’s group with chronic and acute renal insufficiency compared to the other diagnoses. Additionally, the typical “Decoy cells” observed in these samples were more than 10

in a microscopic field, confirming presence of active BKV replication. Pyelonephritis and glomerulonephritis, which are complications after renal transplantation show as well higher number of “Decoy cells”. No “decoy cells” were observed in the samples from control group of patients.

All the patients with chronic and acute renal failure, glomerulonephritis and pyelonephritis were subjected to treatment with anti-rejection drugs Mycophenolat mofetil and/or other corticosteroids, which could be potentially immunosuppressive, thus provoking active viral replication. The other conditions, where the use of antibiotics may lower the immune response also show shedding of “Decoy cells” in some cases. The connection between the clinical status and the etiology of the disease needs to be elucidated with much more sensitive techniques but in all these cases BKV could be a potential inductor of above-presented statuses. These results are in accordance with those received by other authors (9).

Our results show that the search for “Decoy cells” could be applied in the screening of the BK polyomavirus and especially when a rapid diagnose is needed. In fact there is no general algorithm for BKV diagnosis and some authors accept urine cytology and PCR as first line of methods, applied in the routine practice (5, 10). “Decoy cells”, which are caused by BKV infection of the urinary epithelial cells, can be identified by light microscopy. They are often present before BKV disease occurs. The combined presence of inflammatory sediment and “Decoy cells” with allograft dysfunction is described as a more specific marker for BKV infection. Priorities of the urine cytology are the high predicting value, easiness and low cost (6).

From the two human pathogens from the polyomaviridae family BK virus plays major role of the cytology of the urinary tract. In permissive infections BK produces large, homogenous basophilic nuclear inclusions that occupy nearly the entire volume of the enlarged nucleus. Occasionally a very narrow rim of clearing separates the inclusion from the nuclear envelope. The infected cells are usually enlarged and contain only a single nucleus. With the passage of time, the inclusions become less basophilic and acquire a pale, homogenous appearance. The inclusions may also dissolve, leaving out a peculiar network of coarse nuclear chromatin that as diagnostic of the infection as the classical inclusion (7). The presence of “Decoy cells” in urine is a morphological marker for viral replication (10).

The cells bearing viral polyomavirus inclusions are very typical and differ from all other cythopatic effects, induced by viral agents. For example other viruses - the herpesviruses (including CMV, Herpes simplex-1, Herpes simplex-2) and papillomaviruses are also causative agents of specific cythopatic effects of the urothelial cells. The CMV causes very characteristic changes which can be readily recognized in the urinary sediment. The principal abnormality is large, usually basophilic inclusion, surrounded by a large clear zone. There is typical distinct outer belt of condensed chromatin. As these inclusions are very similar to an eye of a bird, they bear the name owl’s or bird’s eye. The other agents- HSV-1 and HSV-
2 are also involved in the pathology of the urinary tract. In the herpesvirus infected cells multinucleation is commonly observed, presumably the result is cell fusion in the presence of the virus. The multiple nuclei are often densely packed, with resulting nuclear molding, recognized by tightly fitting contours of the adjacent cells. The papillomaviruses, which cause venereal warts, may also occur in the lower urinary tract, mainly in the urethra and the urinary bladder and may cause significant cytologic abnormalities—koilocytosis (7).

Conclusions
BK virus is an emerging pathogen, which is known to cause severe infections in immunocompromised patients, and especially those subjected to bone-marrow and renal transplantation. In renal allograft patients it is connected with the polyomavirus associated nephritis (PVAN) but in bone marrow with hemorrhagic cystitis. There is general consensus that immunosuppression is the most important factor for emerging of PVAN. The allograft failure is the most serious complication after transplantation. It is fully reversible early in the disease, although persistent viral damage leads to fibrosis and tubular atrophy. Thus, establishing proper instant diagnosis is critical for the management of the transplant patients and applying the proper therapy scheme. From the different methods, urine cytology serves as a promising tool, when assessing the patient’s condition not only because of its low cost but also the high predicting value.

REFERENCES

TABLE 1
Distribution of the patients according their diagnoses

<table>
<thead>
<tr>
<th>Diagnose</th>
<th>Number of patients</th>
<th>Year rage</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Renal allograft patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyelonephritis (chronic, obstructive, acute, recurrens)</td>
<td>19</td>
<td>18-68</td>
<td>8</td>
</tr>
<tr>
<td>Chronic renal insufficiency</td>
<td>17</td>
<td>14-70</td>
<td>9</td>
</tr>
<tr>
<td>Glomerulonephritis (chronic, membranous, mesangioproliferative)</td>
<td>17</td>
<td>21-67</td>
<td>5</td>
</tr>
<tr>
<td>Acute renal insufficiency</td>
<td>2</td>
<td>49, 58</td>
<td>2</td>
</tr>
<tr>
<td><strong>B. Other urologic disorders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydronephrosis</td>
<td>1</td>
<td>48</td>
<td>-</td>
</tr>
<tr>
<td>Tumor renalis dexter</td>
<td>1</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Status post TUTUR</td>
<td>1</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>Status post adenomectomia</td>
<td>4</td>
<td>59-75</td>
<td>2</td>
</tr>
<tr>
<td>Status post pyelolithotomia</td>
<td>1</td>
<td>55</td>
<td>-</td>
</tr>
<tr>
<td>Stricture urethrae</td>
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<td>28, 31</td>
<td>1</td>
</tr>
<tr>
<td>Status post diverticulotomia</td>
<td>1</td>
<td>66</td>
<td>1</td>
</tr>
<tr>
<td>Prostatitis chronica</td>
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<td>34</td>
<td>-</td>
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<tr>
<td>Unidentified renal infections</td>
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<td>10-68</td>
<td>3</td>
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<td><strong>C. Control group</strong></td>
<td>10</td>
<td>25-50</td>
<td>-</td>
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