The Value of Urinary Decoy Cells Finding in Patients with Kidney Transplantation

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ABSTRACT

Childhood infection with polyomaviruses leads to a life-long latent infection of renal and urinary tract epithelia. Replication in the reno-urinary epithelium is associated with viral cytopathic changes such as nuclear inclusions and decoy cells. During the 2005-2009 period, cytological urine analysis was performed in 154 samples (94 male and 60 female) from patients with kidney transplantation (n=19), simultaneous pancreas-kidney transplantation (SPKT) (n=9) and simultaneous kidney and liver transplantation (n=2). Urine samples were analyzed monthly following transplantation according to the protocol. The period from transplantation to the first occurrence of decoy cells in the urine and the period of decoy cell persistence in the urine were assessed. The presence of decoy cells (<10 and >10 decoy cells) and red blood cells (<20 E, 20-100 E and >100 E) per cytospin smear was semiquantitatively determined, along with analysis of inflammatory cells (neutrophilic granulocytes) and fungi. In patients with decoy cells detected, their sensitivity, specificity, and negative and positive predictive value for BK virus nephropathy were calculated. Correlation of the study parameters was estimated by use of Kruskal-Wallis test (Statistica 7.1, StatSoft Inc., Tulsa, USA). Decoy cells were found in 30 patients (20 male and 10 female), age median 40 (range 16-69) years, at a mean of day 115 (range day 5–747) post transplantation, whereas their presence was recorded for a mean of 141 (range 77–771) days. Immunohistochemical staining of kidney biopsy sample for polyomavirus (SV40 large T-antigen) yielded positive reaction in 2/30 (7%) patients. Erythrocyturia was present in 29/30 patients with decoy cells. The number of decoy cells per cytospin smear generally ranged less than 10 in 25/30 patients, whereas more than 10 decoy cells per cytospin smear were only recorded in 5/30 patients. Immunohistochemistry produced positive finding for BK virus in one patient with SPKT and simultaneous kidney and liver transplantation each, which was statistically significantly more common as compared with patients with kidney transplantation alone (p=0.0244). Immunohistochemical positivity for BK virus was more significant in cases with more than 10 decoy cells detected in cytospin smear (p=0.013). In BK nephropathy, the finding of urinary decoy cells showed a 100% sensitivity, 84% specificity, 100% negative predictive value and 6% positive predictive value. BK virus nephropathy remains a significant post transplantation complication.

Key words: decoy cells, human polyomavirus, renal transplant recipients

Introduction

Primary polyomavirus infection occurs in early childhood and the virus remains latent in the urinary tract epithelium¹-³. Three polyomavirus species, BK virus (BKV), JC virus (JCV), and simian virus (SV40), cause disease in humans⁴-⁶. BKV nephropathy is one of the most important complications of BKV infection⁷-⁸. Immunosuppression of the allograft recipient can lead to reactivation of the infection and development of nephropathy resulting in allograft failure in up to 1%-5% of kidney transplant recipients⁹. When reactivated, the virus proliferates within the nuclei of renal tubular and urothelial cells producing viral cytopathic effect manifested with nuclear enlargement and basophilic intranuclear inclusions¹⁰,¹¹. Such cells known as »decoy cells« can be identified by urine cytology. Systematic determination of viruria with cytologic or molecular methods has emerged

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as the most useful tool for screening renal transplant recipients as it identifies patients with polyomavirus replication in urinary tract\textsuperscript{12–17}. Tissue biopsy is considered the gold standard for documentation of BKV nephropathy\textsuperscript{12}. The aim of this presentation is to report data on the evaluation of the efficacy of urine cytology in detection of BKV in renal transplant recipients.

Materials and Methods

During the 2005–2009 period, cytologic analysis of urine was performed in 154 samples (94 male and 60 female) of patients with transplanted solid organs. In 30 cases, decoy cells were present in voided urine. The majority of patients had undergone kidney transplantation (n=19), then simultaneous pancreas-kidney transplantation (SPKT; n=9), and simultaneous kidney-liver transplantation (n=2). Urine samples were analyzed monthly after transplantation, according to the protocol. Early in the morning the patient voided the urine collected in the urinary bladder overnight; the next fresh urine sample was referred to cytology laboratory within 15 minutes of miction; 0.5–1 mL of urine was processed in a cytocentrifuge at 600 rpm for 5 minutes. Slides were air-dried for May-Grünwald-Giemsa (MGG) staining, or immediately fixed in 95% alcohol for Papanicolaou staining. Two slides of each urine sample were analyzed, one stained with MGG and Papanicolaou staining method each. Time interval between the day of transplantation and first appearance of decoy cells in the urine and period of decoy cell persistence in the urine were assessed. Also, the presence of red blood cells (E) per cytospin slide (<20 E, 20–100 E, and >100 E), and the presence of inflammatory cells (neutrophilic granulocytes) and fungi was semiquantitatively determined. Kidney biopsy was performed according to the protocol (40%) or clinical indication (53%), or immediately after transplantation (time zero biopsy; 7%). Acute cellular rejection (ACR), acute antibody mediated rejection, chronic rejection, acute tubular injury (ATI), interstitial fibrosis and tubular atrophy (IF and TA), blood vessel changes, and presence of polyomavirus (PV) on light microscopy and on immunohistochemical staining (Anti-SV40 T antigen, clone PAb416, Calbiochem) were analyzed in biopsy specimens. The presence of other kidney diseases such as glomerular ones and samples with normal findings (NF) were also noted. Analysis of decoy cell appearance after transplantation in days, total duration of decoy cell presence in the urine and semiquantitative analysis of decoy cell presence was performed (<10 DC and >10 DC per smear). In patients with decoy cells detected in urine samples, their sensitivity, specificity, and positive and negative predictive value for BK nephropathy were calculated. Correlation of the study parameters was estimated by use of non-parametric Kruskal-Wallis test (Statistica 7.1, StatSoft Inc., Tulsa, USA).

Results

Decoy cells were found in 30/154 (19.5%) patients (20 male and 10 female) (Figure 1). Median age was 40 (range 16–69) years. The mean interval between kidney transplantation and decoy cell occurrence was 115 (range 5–747) days, and mean duration of decoy cell presence was 141 (range 77–771) days. Immunohistochemical staining of kidney biopsy for polyoma virus was positive in 2/30 (7%) patients (Figure 2). ACR was found in 10/30 (33%) and ATI in 9/30 (30%) patients. IF and TA was found in 5/30 (17%) patients. Kidney morphology was normal in 3/30 (10%) patients. One patient had focal segmental glomerulosclerosis (FSGS) and another one had both FSGS and ATI. Erythrocyturia was found in 29/30 patients with decoy cells (Table 1). Less than 20 erythrocytes per cytospin smear were recorded in 14 patients. More pronounced erythrocyturia (>100 E) was found in 8 cases, whereas 20–100 E per cytospin smear were recorded in 7 patients. The number of decoy cells per smear was less than 10 in most patients, while more than 10 decoy cells were only found in 5 samples. There was no correlation between the finding of neutrophilic granulocytes and fungi, and the presence of decoy cells. Immunohistochemical positivity for BKV was recorded in one patient with SPKT and another one with simultaneous liver and kidney transplantation, which was statistically significant as compared with patients with kidney transplanta-

![Fig. 1. Decoy cells with large hyperchromatic homogeneous nuclear inclusions in urinary sediment: a) May-Grünwald-Giemsa, x1000, b) Papanicolaou x1000.](image-url)
tion alone (p=0.0244). A more significant immunohistochemical positivity was recorded in cytospin smears with more than 10 decoy cells (p=0.013). In BKV nephropathy, the finding of urinary decoy cells showed a 100% sensitivity, 84% specificity, 100% negative predictive value and 6% positive predictive value.

Discussion

Infections with human polyomaviruses types JC and BK are widespread, but the majority of affected patients are asymptomatic. The major clinical manifestations appear to result from reactivation disease in immunocompromised individuals. Although both JCV and SV40 have been implicated in some cases of polyomavirus nephropathy, most cases seem to be caused by BKV. Renal transplant recipients receiving immunosuppressive therapy have a 10–60% chance of polyomavirus reactivation accompanied by shedding of urothelial cells. In our study, 19.5% (30/154) of patients had positive urinary decoy cell findings, which is comparable with literature reports, e.g., Hayat et al. 35% and Drachenberg et al. 13.8%. Viral replication begins early after transplantation and progresses through detectable stages of viruria followed by viremia and nephropathy. Viruria can be detected by polymerase chain reaction (PCR) for BKV DNA, reverse transcription-PCR for BKV RNA, cytology for BKV inclusion bearing epithelial cells termed ‘decoy cells’, or electron microscopy for viral particles. These tests are sensitive for detecting active BKV infections but lack specificity for nephropathy because the detected virus could originate anywhere along the urinary tract. Therefore, transplant kidney biopsy remains the gold standard for diagnosing BKV nephropathy. However, in renal biopsy specimens it is often difficult to differentiate between the tissue effects of viral pathology and changes caused by ACR. The decrease in immunosuppression needed to treat infection is opposite to the increases that are needed to treat rejection. Both exfoliative cytology and quantitation of viruria by PCR can be used in screening renal transplant recipients, which can aid in the identification of patients at risk of developing polyomavirus nephropathy. Molecular tests are more sensitive than urine cytology demonstrating viruria in 30% of samples from renal transplant patients versus 12–16% of cytology samples displaying decoy cells. However, the proportion of patients with viruria identified by urine cytology is closer to the number of patients with overt disease.
that develop polyomavirus nephropathy (8–10%). Hayat et al. demonstrated histologically verified BKV nephropathy in 7% of transplanted patients, whereas Drachenberg et al. report on the incidence of BKV and JCV nephropathy of 5.5% and 0.9%, respectively. These data correspond to our result on 1.3% of transplanted patients with polyomavirus nephropathy. Nickeleit et al. report on the positive predictive value of 'positive' decoy cell analysis to predict BKV nephropathy to be 25–30%. However, the negative predictive value was greater than 99%, i.e. ‿negativeŠ decoy cell analysis indicated absence of viral nephropathy. De Las Casas et al. report on the sensitivity of 83% and specificity of 90%, with a positive predictive value of 63% and negative predictive value of 96% of urine cytology in detecting human polyomavirus compared with electron microscopy of urine samples. The high sensitivity and specificity with a high negative predictive value and low positive predictive value are consistent with our results.

**Conclusion**

Urine cytology is a safe, noninvasive and sensitive tool for the evaluation and follow-up of renal transplant recipients and can be used as prospective screening for BKV allograft nephropathy.
SAŽETAK

Infekcija poliomavirusu tijekom djetinjstva uzrokuje doživotnu latentnu infekciju epitelita bubrega i mokraćnog sustava. Replikacija virusa u epitelijalnim stanicama bubrega i mokraćnog sustava udružena je s virusnim citopatskim promjenama (nuklearne inkluzije) i virurijom (decoy stanica, virioni i/ili virusni proteini u mokraći). U razdoblju od 2005. do 2009. godine ciljevna analiza močvara učinjena je kod 154 bolesnika (94 muškaraca i 60 žena) s transplantiranim solidnim organima. U uzorcima 30 bolesnika nađene su decoy stanice. Uglavnom se radilo o transplantiranim bubrezima (19 bolesnika), istodobnoj transplantaciji guštera i bubrega (SPKT) kod 9 bolesnika, a u 2 slučajeva se radilo tijekom solidnih organskih transplantacija. U 30 bolesnika nađene su decoy stanice. Uglavnom se radilo o transplantiranim bubrezima (19 bolesnika), istodobnoj transplantaciji guštera i bubrega (SPKT) kod 9 bolesnika, a u 2 slučajeva se radilo tijekom solidnih organskih transplantacija. U 30 bolesnika nađene su decoy stanice. U 30 bolesnika nađene su decoy stanice.