Coll. Antropol. **34** (2010) 1: 153–157
Original scientific paper

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

Željko Vidas[1], Maja Mišić[2], Arijana Pačić[3], Franjo Jurenec[1], Mladen Knotek[1,2] and Ika Kardum-Skelin[1,2]

[1] University Hospital »Merkur«, Zagreb, Croatia
[2] Department of Cytology, General Hospital »Dr. J. Benčević«, Slavonski Brod, Croatia
[3] Department of Pathology, University Hospital Dubrava, Zagreb, Croatia
[4] University of Zagreb, School of Medicine, Zagreb, Croatia

A B S T R A C T

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 epithelium[1–3]. Three polyomavirus species, BK virus (BKV), JC virus (JCV), and simian virus (SV40), cause disease in humans[4–6]. BKV nephropathy is one of the most important complications of BKV infection[7,8]. 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 recipients[9]. 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 inclusions[10,11]. Such cells known as »decoy cells« can be identified by urine cytology. Systematic determination of viruria with cytologic or molecular methods has emerged

Received for publication September 18, 2009

as the most useful tool for screening renal transplant recipients as it identifies patients with polyomavirus replication in urinary tract[12–17]. Tissue biopsy is considered the gold standard for documentation of BKV nephropathy[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-

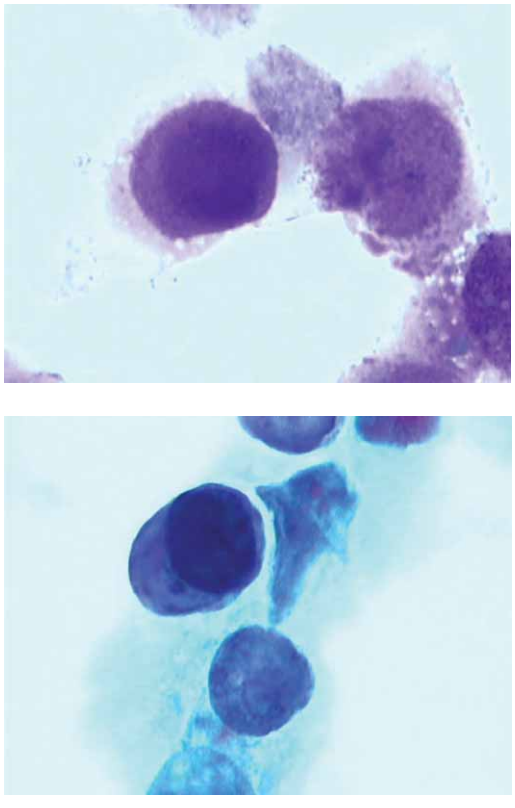

Fig. 1. Decoy cells with large hyperchromatic homogeneous nuclear inclusions in urinary sediment: a) May-Grûnwald-Giemsa, x1000, b) Papanicolaou x1000.

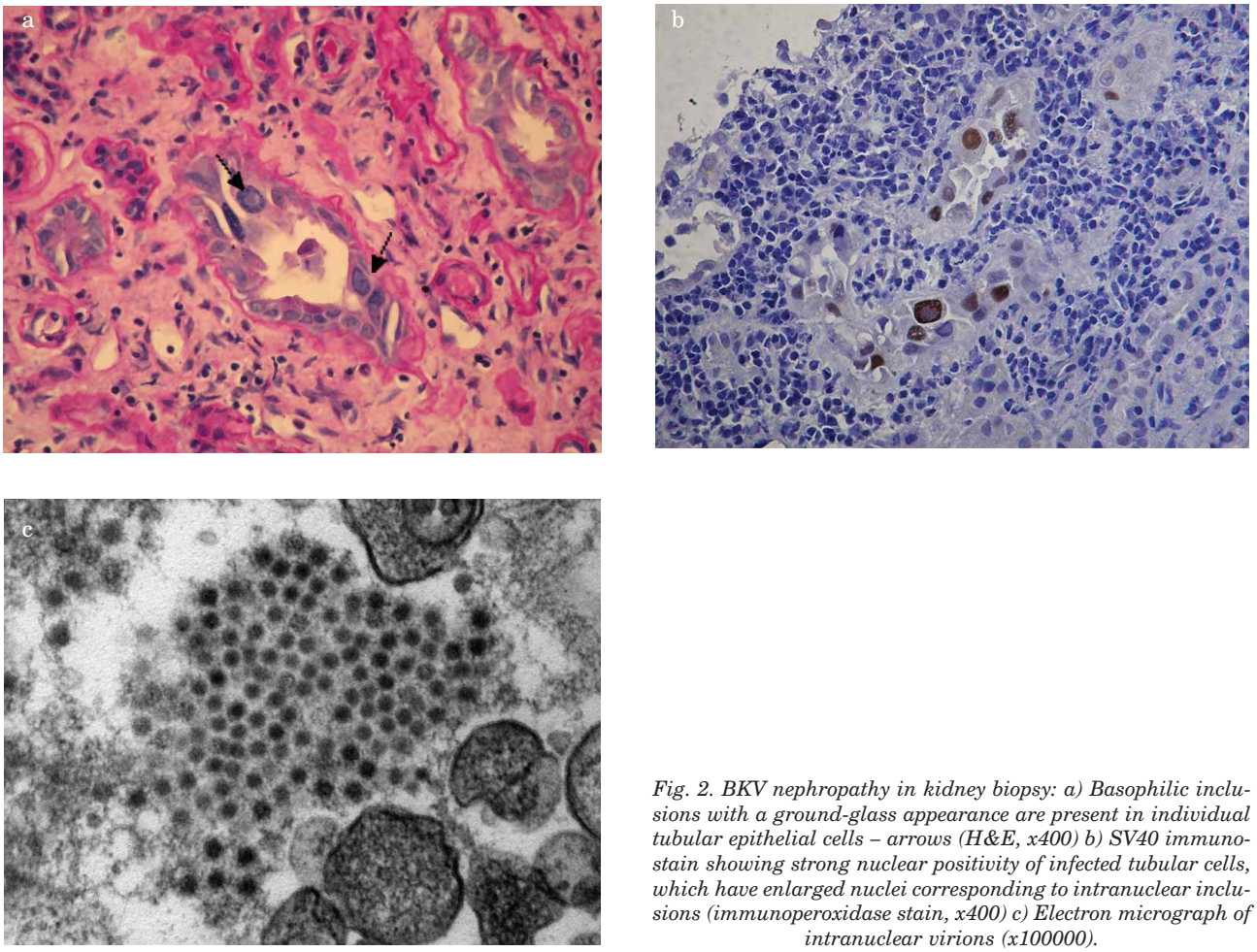

Fig. 2. BKV nephropathy in kidney biopsy: a) Basophilic inclusions with a ground-glass appearance are present in individual tubular epithelial cells – arrows (H&E, x400) b) SV40 immunostain showing strong nuclear positivity of infected tubular cells, which have enlarged nuclei corresponding to intranuclear inclusions (immunoperoxidase stain, x400) c) Electron micrograph of intranuclear virions (x100000).

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[18]. Although both JCV and SV40 have been implicated in some cases of polyomavirus nephropathy, most cases seem to be caused by BKV[7]. Renal transplant recipients receiving immunosuppressive therapy have a 10–60% chance of polyomavirus reactivation accompanied by shedding of urothelial cells[19]. 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.[20] 35% and Drachenberg et al.[21] 13.8%. Viral replication begins early after transplantation and progresses through detectable stages of viruria followed by viremia and nephropathy[6,13,22–25]. 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 patricles[6,8,13,22,26]. 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[6,27]. 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[28]. The decrease in immunosuppression needed to treat infection is opposite to the increases that are needed to treat rejection[6]. 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[14,29,30]. 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[29,30]. However, the proportion of patients with viruria identified by urine cytology is closer to the number of patients

TABLE 1
DATA OF PATIENTS WITH DECOY CELLS IN URINE SEDIMENT

	Patient	Sex	Age (yrs)	Histopathology	TB	KBT	TO	DAT	PDT	NDC	NE	F
1	B V	M	45	ACR	P	195	SPKT	200	119	5	6	1
2	B A	M	41	ATI	0	0	SPKT	7	1	1	5	1
3	C A	M	56	NF	P	120	SPKT	124	1	2	8	1
4	C M	F	57	ATI, FSGS	P	90	SPKT	192	49	10	27	1
5	D Ž	M	39	NF	P	30	SPKT	375	88	1	101	1
6	G B	F	39	FSGS	I	15	SPKT	148	1	1	2	2
7	H Ž	M	57	ACR	I	105	K	94	117	65	4	2
8	H Ž	F	57	IF, TA	I	150	K	102	475	1	3	1
9	I Z	M	48	ACR	I	225	K	213	1	54	101	1
10	J B	M	36	ATI	P	90	K	178	1	22	3	1
11	K Z	M	69	ATI	I	18	K	394	7	1	8	1
12	K I	M	19	PV	I	375	K+L	346	74	16	4	1
13	L D	M	57	NF	P	90	K	36	61	2	9	1
14	L F	M	31	PV	I	105	SPKT	153	771	26	29	1
15	M B	M	35	IF, TA	P	365	K	747	1	1	4	1
16	M D	M	42	ACR	P	90	K	72	164	6	37	1
17	M L	M	30	ACR	I	120	K	198	1	1	101	2
18	M T	F	27	ATI	I	47	SPKT	150	290	3	4	2
19	N A	F	48	ATI	P	180	K	603	257	5	5	1
20	P D	M	32	ACR	I		K	210	1	1	41	1
21	P Z	F	51	ATI	I	480	K	463	80	1	93	1
22	Q B	M	38	ACR	I	30	K	7	355	6	101	1
23	S S	F	26	ACR	P	90	SPKT	85	1	1	26	1
24	S Z	F	34	ATI	I	4	K	5	191	3	101	1
25	S M	M	67	IF, TA	P	90	K	125	1	2	101	1
26	S S	F	31	IF, TA	0	0	K	364	334	3	22	2
27	Š M	F	16	ACR	P	365	K	367	477	2	101	1
28	Š M	M	47	ATI	I	21	K	20	199	2	11	1
29	Š A	M	41	IF, TA	I		K	88	1	1	9	1
30	V L	M	31	ACR	I	22	K+L	7	114	4	101	1
							$p<0.05$			$p<0.05$		

TB – type of biopsy, P – protocol biopsy, I – indicated biopsy, 0 – time zero biopsy, DAT – decoy cell appearance after transplantation (in days), PDT – presence of decoy cells (in days), TO – transplanted organs, SPKT – simultaneous pancreas-kidney transplantation, K – kidney, L – liver, NDC – number of decoy cells *per* cytospin smear, NE – number of erythrocytes *per* cytospin smear, Histopathology – histopathological diagnosis of kidney biopsy, ACR – acute cellular rejection, ATI – acute tubular injury, IF – interstitial fibrosis, TA – tubular atrophy, FSGS – focal segmental glomerulosclerosis, KBT – kidney biopsy after transplantation (in days), PV – polyomavirus, F – fungi (1 – not present; 2 – present)

that develop polyomavirus nephropathy (8–10%)[13,29]. Hayat et al.[20] demonstrated histologically verified BKV nephropathy in 7% of transplanted patients, whereas Drachenberg et al.[21] 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.[31] 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. »negative« decoy cell analysis indicated absence of viral nephropathy. De Las Casas et al.[32] 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.

REFERENCES

1. BROWN P, TSAI T, GAJDUSEK DC, Am J Epidemiol, 102 (1975) 331. — 2. MANNON RB, Transplantation, 77 (2004) 1313. — 3. RANDHAWA P, DEMETRIS A, N Engl J Med, 342 (2000) 1361. — 4. KNOWLES WA, PIPKIN P, ANDREWS N, VYSE A, MINOR P, BROWN DW, MILLER E, J Med Virol, 71 (2003) 115. — 5. STOLT A, SASNAUSKAS K, KOSKELA P, LEHTINEN M, DILLNER J, J Gen Virol, 84 (2003) 1499. — 6. BOHL DL, BRENNAN DC, Clin J Am Soc Nephrol, 2 (2007) 36. — 7. BANVOISIN C, WEEKERS L, XHIGNESSE P, GROSCH S, MILICEVIC M, KRZESINSKI J, Transplantation, 85 (2008) 42. — 8. GARDNER SD, MACKENZIE EF, SMITH C, PORTER AA, J Clin Pathol, 37 (1984) 578. — 9. HIRSCH HH, Am J Transplant, 2 (2002) 25. — 10. TRAYSTMAN MD, GUPTA PK, SHAH KV, REISSIG M, COWLES LT, HILLIS WD, FROST JK, Acta Cytol, 24 (1980) 501. — 11. KAHAN AV, COLEMAN DV, KOSS LG, Am J Clin Pathol, 74 (1980) 326. — 12. HIRSCH HH, BRENNAN DC, DRACHENBERG CB, GINEVRI F, GORDON J, LIMAYE AP, MIHATSCH MJ, NICKELEIT V, RAMOS E, RANDHAWA P, SHAPIRO R, STEIGER J, SUTHANTHIRAN M, TROFE J, Transplantation, 79 (2005) 1277. — 13. HIRSCH HH, KNOWLES W, DICKENMANN MM, PASSWEG J, KLIMKAIT T, MIHATSCH MJ, STEIGER J, N Engl J Med, 347 (2002) 488. — 14. DRACHENBERG RC, DRACHENBERG CB, PAPADIMITRIOU JC, RAMOS E, FINK JC, WALI R, WEIR MR, CANGRO CB, KLASSEN DK, KAHKED A, CUNNINGHAM R, BARTLET ST, Am J Transplant, 1 (2001) 373. — 15. DRACHENBERG CB, PAPADIMITRIOU JC, HIRSCH HH, WALI R, CROWDER C, NOGUEIRA J, CANGRO CB, MENDLEY S, MIAN A, RAMOS E, Am J Transplant, 4 (2004) 2082. — 16. DRACHENBERG CB, HIRSCH HH, RAMOS E, PAPADIMITRIOU JC, Hum Pathol, 36 (2005) 1245. — 17. NICKELEIT V, MIHATSCH MJ, Am J Transplant, 4 (2004) 838. — 18. DEMETER LM, Clinical manifestations and diagnosis of JC, BK and other polyomavirus infections, UpToDate, accessed 27.8.2009. Available from: URL: http://www.uptodate.com/patients/content/topic.do?topicKey=č2Go6D5Whe7RIhnb. — 19. RANDHAVA PS, FINKELSTEIN S, SCANTLEBURY V, SHAPIRO R, VIVAS C, JORDAN M, PICKEN MM, DEMETRIS AJ, Transplantation, 67 (1999) 103. — 20. HAYAT A, MUKHOPADHYAY R, RADHIKA S, SACHDEVA MS, NADA R, JOSHI K, SAKHUJA V, JHA V, Nephrology, 13 (2008) 157–163. — 21. DRACHENBERG CB, HIRSCH HH, PAPADIMITRIOU JC, GOSERT R, WALI RK, MUNIVENKATAPPA R, NOGUEIRA J, CANGRO CB, HARIRIAN A, MENDELY S, RAMOS E, Transplantation, 84 (2007) 323. — 22. BRENNAN DC, AGHI I, BOHL DL, SCHNITZLER MA, HARDINGER KL, LOCKWOOD M, TORRENCE S, SCHUESSLER R, ROBY T, GAUDREAULT-KEENER M, STORCH GA, Am J Transplant, 5 (2005) 582. — 23. BRESSOLLETTE-BODIN C, COSTE-BUREL M, HOURMANT M, SEBILLE V, ANDRE-GARNIER E, IMBERT-MARCILLE BM, Am J Transplant, 5 (2005) 1926. — 24. LIMAYE AP, JEROME KR, KUHR CS, FERRENBERG J, HUANG ML, DAVIS CL, COREY L, MARSH CL, J Infect Dis, 183 (2001) 1669. — 25. NICKELEIT V, KLIMKAIT T, BINET IF, DALQUEN P, DEL ZENERO V, THIEL G, MIHATSCH MJ, HIRSCH HH, N Engl J Med, 342 (2000) 1309. — 26. DING R, MEDEIROS M, DADHANIA D, MUTHUKUMAR T, KRACKER D, KONG JM, EPSTEIN SR, SHARMA VK, SESHAN SV, LI B, SUTHANTHIRAN M, Transplantation, 74 (2002) 987. — 27. RANDHAVA P, HO A, SHAPIRO R, VATS A, SWALSKY P, FINKELSTEIN S, UHRMACHER J, WECK K, J Clin Microbiol, 42 (2004) 1176. — 28. KAPILA K, NAMPOORY MRN, JOHNY KV, PACSA AS, AL-AYADHY B, MATHEW JR, NAIR MP, HALIM MA, GEORGE SS, FRANCIS IM, Med Princ Pract, 16 (2007) 237. — 29. DRACHENBERG CB, PAPADIMITRIOU JC, Transpl Infect Dis, 8 (2006) 68. — 30. RANDHAWA P, HO A, SHAPIRO R, Transplantation, 79 (2005) 984. — 31. NICKELEIT V, HIRSCH HH, BINET IF, GUDAT F, PRINCE O, DALQUEN P, THIEL G, MIHATSCH MJ, J Am Soc Nephrol, 10 (1999) 1080. — 32. DE LAS CASAS LE, HOERL HD, BARDALES RH, PIRSCH JD, SEMPF JM, WETZEL DJ, STEWART III J, OBERLEY TD, KURTYCZ DFI, Diagn Cytopathol, 25 (2001) 376.

VRIJEDNOST NALAZA DECOY STANICA U MOKRAĆI BOLESNIKA S TRANSPLANTIRANIM BUBREGOM

SAŽETAK

Infekcija poliomavirusom tijekom djetinjstva uzrokuje doživotnu latentnu infekciju epitela bubrega i mokraćnog sustava. Replikacija virusa u epitelnim stanicama bubrega i mokraćnog sustava udružena je s virusnim citopatskim promjenama (nuklearne inkluzije) i virurijom (decoy stanice, virioni i/ili virusni proteini u mokraći). U razdoblju od 2005. do 2009. godine citološka analiza mokraće 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 gušterače i bubrega (SPKT) kod 9 bolesnika, a u 2 slučaja se radilo o istodobnoj transplantaciji bubrega i jetre. Obrađivali su se svježi uzorci mokraće unutar 15 min od uzimanja (ne prvi jutarnji uzorak); 0,5 mL mokraće je centrifugirano u citocentrifugi na 600 okretaja kroz 5 minuta, a preparati su bojani metodom po May-Grünwald-Giemsi (MGG) i Papanicolaou. Analiziralo se razdoblje od transplantacije do prve pojave decoy stanica u mokraći i razdoblje prisutnosti decoy stanica u mokraći. Semikvantitativno se procjenjivala prisutnost decoy stanica (<10 DC i >10 DC) i prisutnost eritrocita (<20 E, 20–100 E i >100 E) po citospin razmazu, a analizirao se i nalaz upalnih stanica (neutrofilnih granulocita) i gljiva. U bolesnika kod kojih su nađene decoy stanice izračunavala se osjetljivost, specifičnost te negativna i pozitivna prediktivna vrijednost na BKV nefropatiju. Izvršena je međusobna korelacija ispitivanih pokazatelja Kruskal-Wallis testom (Statistica 7.1, StatSoft Inc., Tulsa, SAD. Decoy stanice su nađene kod 30 bolesnika (20 muškaraca i 10 žena), s medijanom dobi od 40 (16–69) godina, prosječno nakon 115. dana od transplantacije (5.–747. dan), a prisutnost je zabilježena prosječno tijekom 141 dana (77.–771. dan). Imunohistokemijskim bojenjem bioptičkog uzorka bubrega na poliomavirus (SV40 large T-antigen) pozitivna reakcija nađena je u 2/30 (7%) bolesnika. Eritrociturija je bila prisutna kod 29/30 bolesnika s decoy stanicama. Broj decoy stanica po razmazu se uglavnom kretao do 10, a u svega 5 uzoraka je broj decoy stanica prelazio 10 po citospin razmazu. Imunocitokemijski pozitivan nalaz na BKV nađen je u jednog bolesnika sa SPKT te u jednog bolesnika s istodobnom transplantacijom jetre i bubrega, što je statistički značajno prema bolesnicima kod kojih je transplantiran samo bubreg ($p=0,0244$). Imunohistokemijska pozitivnost na BKV je bila značajnija ako je u citospin razmazu nađeno više od 10 decoy stanica ($p=0,013$). Nalaz decoy stanica u mokraći kod BKV nefropatija ima 100%-tnu osjetljivost i 84%-tnu specifičnost te negativnu prediktivnu vrijednost od 100% i pozitivnu prediktivnu vrijednost od 6%. Citološka analiza mokraće je osjetljiva i specifična metoda utvrđivanja aktivne infekcije poliomavirusom, značajna u probiru bolesnika s transplantiranim bubrezima i prevenciji BKV nefropatije