ORIGINAL ARTICLE

Urine cytology screening for polyoma virus infection following renal transplantation: the Oxford experience

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J Clin Pathol 2007;**60**:927–930. doi: 10.1136/jcp.2006.042507

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Accepted 13 October 2006
Published Online First
8 December 2006

Objective: To review the first year of a monthly urine cytology screening service, introduced to identify renal transplant patients at risk of polyoma virus nephropathy (PVN), at an early, potentially treatable, stage.

Methods and results: Monthly urine samples (n=392) were received from 97/108 transplant recipients in 2005. Of 56 patients with follow-up >6 months, 20% and 9% had significant (>10 decoy cells/cytospin) and non-significant positive cytology, respectively. The first positive urine samples occurred most commonly in the second and third month post-transplantation and patients with significantly positive samples had higher 3-month and 6-month serum creatinine levels than patients with negative urine cytology (p<0.01). Four patients with positive urine cytology had a subsequent positive plasma BK virus PCR; 3/97 patients had biopsy-proven PVN, all in the third month, 1–6 weeks after first positive urine samples.

Conclusions: Significant PV viruria is common following renal transplantation with onset usually within the first 3 months. Viruria is associated with worse graft function at 3 and 6 months. The time between urine positivity and clinical PVN is short. More frequent early urine screening would be required to achieve clinical benefit.

Polyoma virus (PV) infection or re-activation is common following renal transplantation, most cases being BK virus (BKV) which is widespread in the general population with seroprevalence rates of 80–90%.^{1,2} In immunocompetent individuals, primary BKV infection of the urinary tract is usually asymptomatic with the virus persisting in a latent state. The first stage of infection/re-activation post-transplantation is defined by subclinical viral replication in the urothelium, occurring in 30–60% of patients.^{3,4} This is diagnosed by urine cytology to demonstrate virally infected cells (decoy cells) or by detection of PV DNA in urine by PCR.^{3,5} This is followed by viraemia in 10–40% of patients.^{3,4} diagnosed by the detection of PV DNA in the plasma, that may reflect preclinical PV nephropathy (PVN) as the infection ascends into renal tubules.⁶ Early PVN is focal and renal biopsy may not show evidence of infection.⁷ Finally, there is clinical PVN with renal dysfunction and a viral nephritis on histology, affecting 1–10% of renal transplant recipients.^{3,8–10} This is associated with irreversible injury and progressive graft failure in up to 70% of cases.^{3,6,9,11–13}

PVN is always preceded by urinary reactivation, the median time from urine positivity to PVN diagnosis being 12 weeks in the series of Hirsch *et al.*⁸ The aim of urine screening is to identify those patients at risk of PVN at an early stage, before renal injury and dysfunction, in order to institute appropriate management. Early reduction of immunosuppression may allow clearance of the virus with a low risk of rejection.^{4,7} Criteria for the presumptive diagnosis of pre-clinical PVN have been proposed: either histological identification of virus in protocol renal biopsies in the presence of stable graft function, or persistent high viruria (>10⁷ viral copies/ml) or viraemia (>10⁴ viral copies/ml) for >3 weeks, in the presence of a negative biopsy. Current guidelines⁵ recommend at least three-monthly screening for the first two years post-transplantation and annually thereafter until five years. However, our previous experience in Oxford was that the median time to diagnosis of clinical PVN was 6 months post-transplantation, meaning that a three-monthly screening service would provide little clinical benefit. Therefore, when we introduced a urine cytology screening service, the frequency of screening was monthly for

the first year post-transplantation. Here we report the findings of this service, the first to be introduced in the UK, and make recommendations for future PV screening.

MATERIALS AND METHODS

Patients

Urine screening was introduced in March 2005. This study reviews the data from the first 12 months for patients who received renal transplants between January and December 2005.

All patients received induction with Campath (anti-CD52) or basiliximab (anti-CD25), followed by tacrolimus with mycophenolate or azathioprine, with or without prednisolone.

Screening protocol

Patients were to have urine cytology samples monthly up to 12 months post-transplantation. Decoy cells were quantified on Papanicolou-stained cytopsins, as <5, 5–10 or >10 cells per cytospin, the latter being considered significant.¹⁴

If <10 decoy cells/cytospin were identified and renal function was stable, urine screening continued as before. If >10 decoy cells/cytospin were identified and renal function was stable, serum for BKV PCR was obtained at the next monthly clinic visit. PCR was performed only if the concurrently obtained urine sample again showed >10 decoy cells. Renal biopsies were performed when clinically indicated; there were no protocol biopsies.

Real-time quantitative PCR

Quantitation of BK virus DNA was performed according to published methods.^{15,16} Briefly, nucleic acid was extracted from 200 µl serum using the QIAamp DNA blood kit (Qiagen, Hilden, Germany), and eluted into 200 µl buffer. The real-time PCR reaction, specific for BK virus, was carried out on an ABI Prism 7000 sequence detector (Applied Biosystems, Warrington UK). The detection limit was 200 genome copies/ml.

Abbreviation: PVN, polyoma virus nephropathy

Figure 1 (A) Cytopsin preparation of a urine sample with abundant decoy cells (Papanicolaou stain, ×200). (B) A decoy cell showing an enlarged nucleus with a basophilic intranuclear inclusion (Papanicolaou stain, ×400).

RESULTS

A total of 392 urine samples were obtained from 97 of 108 patients transplanted in 2005. The mean number of urine samples/patient was 4.04 (range 1–12). Follow-up ranged from 1 to 13 months. Fifteen and 9 patients had significant and non-significant positive urine samples, respectively (fig 1).

Fifty-six patients had follow-ups of at least 6 months, mean follow-up 9.3 months (range 6–13 months). For this cohort, the mean number of urine samples/patient was 5.16 (range 1–12). Eleven of 56 patients (20%) had at least one significant positive urine specimen and 5/56 (9%) had at least one non-significant positive urine. Donor type, HLA-matching and immunosuppression did not differ between groups (table 1).

Figure 2 Graph showing the month post-transplant of the first significant positive urine sample for patients with at least 6 months follow-up.

There was a non-significant trend towards greater delayed graft function (requiring post-transplant dialysis) in patients with significant positive urine samples (45.5%) compared with those with negative urine samples (17.5%).

Of the 56 patients with >6 months follow-up, the first urine sample was obtained in the first month post-transplant in 20 patients, and in the second month in 16 patients; 30 patients had their first urine samples obtained in the third to sixth months post-transplant. Four of 11 patients with significant positive urine samples, had their first significant positive urine sample in the second month post-transplant and 4 patients in the third month (fig 2). In 5 of these 11 patients (45.5%), the first urine sample examined showed a significant positive result. The earliest significant positive sample occurred in the fifth week post-transplant.

The 11 patients with significant positive urine samples had significantly higher serum creatinine concentrations at 3 and 6 months post-transplantation compared with the 40 patients with negative samples (table 1).

Table 1 Graft characteristics and function in patients with at least 6 months follow-up				
	Significant positive*	Non-significant positive*	Negative*	p-value†
Number of patients	11	5	40	
Cadaveric kidneys	10	4	37	n.s.
Mean number of HLA mismatches	3.27	2.6	2.68	n.s.
Post-transplant dialysis	5	0	7	n.s.
Campath induction	7	3	24	n.s.
Basiliximab induction	4	2	16	n.s.
MMF‡	9	5	38	n.s.
Azathioprine§	2	0	2	n.s.
3-month mean serum creatinine	182	145	134	0.0025
3-month median serum creatinine (range)	165 (107–358)	134 (104–218)	123 (83–278)	
6 month mean serum creatinine	183	151	131	0.0014
6-month median serum creatinine (range)	151 (98–347)	133 (108–251)	129 (86–250)	

*Refers to urine cytology result; †Refers to comparison between significant positive and negative cases (X² and Mann–Whitney U test); ‡Mycophenolate mofetil included in baseline immunosuppression; §Azathioprine included in baseline immunosuppression; n.s.: not significant.

Follow-up of patients with positive urine cytology

Fifteen of the 97 patients with urine samples had significant positive urine cytology, 7 of whom had plasma PCR testing, as dictated by protocol (table 2). PCR was positive in 4 patients, viral copy numbers ranging from 2035 to 240 446 copies/ml. Six of the 15 patients with significant positive urine cytology had renal biopsies (table 2); three showed histological PVN, confirmed by positivity for SV40 T-antigen. One of these also showed concurrent tubulo-interstitial rejection. Biopsies in the other three patients showed chronic changes with superimposed acute tubular damage in one. Immunohistochemistry for SV40 T-antigen was negative in these biopsies.

The three biopsies showing PVN were obtained 1, 5 and 6 weeks after the first significant positive urine samples. Two of the three patients with PVN had positive plasma PCR for BKV but the results were obtained after the biopsies were performed. The third patient with PVN had a negative PCR, performed 4 weeks prior to the biopsy diagnosis.

No patient with a negative or non-significant positive urine cytology developed PVN. Immunohistochemistry for SV40 T-antigen is performed as routine on all our renal transplant biopsies, ensuring that no PVN is missed.

Patients with significant positive urine samples were treated in a variety of ways, as the screening programme did not include management protocols (table 2). Five of 15 patients had no treatment, four had reduction of immunosuppression alone, three had reduction of immunosuppression plus a 10-day

Table 2 Follow-up of patients with significant positive urine cytology (n = 15).

Patient number	Month of 1 st positive urine sample	No. of previous negative urine samples	Renal function after 1 st positive urine sample	Treatment based on positive urine result	Time to negative urine (months)	PCR indicated by protocol/done/results	Biopsy result if biopsy done	Time from first positive urine to biopsy
1	4	0	Stable	Nil	2	N*	Acute tubular injury	6 weeks
2	2	0	Stable	Nil	1	N		
3	3	0	Stable	IS reduced	3	Y/N		
4	2	1	Stable	IS reduced	1.5	Y/Y/Neg		
5	4	0	Stable	IS reduced	Died from PTLT	N	No AR. No PVN. No PTLT.	5 weeks
6	3	1	Stable	IS reduced; ciprofloxacin	5.5	Y/Y/Pos		
7	7	5	Stable	IS reduced; ciprofloxacin	Still Pos after 4 months	Y/Y/Pos		
8	3	1	Stable	Nil	1	N		
9	2	0	Worsening	Nil	3	Y/Y/Pos	PVN	5 weeks
10	2	1	Stable	No F/U	No F/U	N		
11	3	2	Stable	IS reduced; ciprofloxacin	Still Pos after 3 months	Y/Y/Neg	Mild changes. No AR	NA
12	2	1	Stable	Ciprofloxacin	Still Pos after 3 months	N*		
13	3	0	Stable	Nil	Still Pos after 3 months	Y/Y/Neg		
14	3	0	Worsening	Nil	Still Pos after 3 months	Y/N	PVN, AR	1 week
15	2	0	Worsening	IS reduced	Still Pos after 2 months	Y/Y/Pos	PVN	6 weeks

IS: immunosuppression; Neg: negative; Pos: positive; F/U: follow-up; AR: acute rejection; PVN: polyoma virus nephropathy; PTLT: post-transplant lymphoproliferative disorder; NA: not applicable, biopsy preceded first positive urine specimen. *Only one month had a significant positive urine cytology result.

course of ciprofloxacin, and two had ciprofloxacin alone. One patient died of post-transplant lymphoproliferative disease soon after the first positive urine sample and the last patient was lost to follow-up. No difference was observed between patients with different management in terms of time to urinary clearance of the virus or development of PVN. Ciprofloxacin was used on the basis of a report that the antibiotic reduced urinary BKV reactivation after haematopoietic stem cell transplantation.¹⁷ Its use in our patients did not accelerate clearance of virus, indeed in one patient it was associated with a log increase in viral load in the blood.

DISCUSSION

Rather than a rigorously controlled trial, this is a review of the first year of a working PV screening service in renal transplant patients. Despite a sample being received for only half the designated time points, viruria was common; 20% and 9% of patients with follow-up periods >6 months had significant and non-significant positive urine cytology, respectively. This incidence is similar to recent studies which found viruria rates of 18%,¹⁴ 21%¹¹ and 30%.⁸ The impact of failing to receive all samples as per protocol is uncertain, but it is likely that the true incidence of viruria in our patient population is higher than 29%. Onset of viruria was early post-transplantation, within the first 3 months in 8/11 patients. This is earlier than reported in a previous study which found a median onset of viruria at 16 weeks post-transplantation.⁸

In almost half the patients (5/11) with significant viruria and >6 months follow-up, the first urine sample was positive, indicating that viruria started even earlier. Therefore, in order to detect the earliest cases of viruria, screening should be performed more frequently early post-transplantation. Recent guidelines recommend three-monthly screening.⁵ Such a protocol, based on our experience, would miss onset of viruria

in most patients, and even the onset of PVN. The early onset of viruria is supported by a study that employed weekly urine PCR and found the median onset of viruria to be 40.5 days.⁴

Patients with significant viruria had worse graft function at 3 and 6 months post-transplantation. This may be cause or effect; whilst tubular injury may predispose to PV re-activation, it is possible that PV infection contributed to graft dysfunction. One possible confounding factor was that patients in the positive group had a higher incidence of post-transplant dialysis. Whilst this finding was not statistically significant, it may have been

Figure 3 Flowchart depicting our recommended protocol for urine cytology screening. (PCR: polymerase chain reaction; EDTA: ethylene diaminetetraacetic acid; IS: immunosuppression; PVN: polyoma virus nephropathy).

clinically significant with some bearing on subsequent renal function.

The rationale for the screening programme was that early reduction in immunosuppression in viraemic patients may reduce the frequency and/or severity of clinical PVN.^{4,5} In our protocol, urine cytology was used to identify those patients who are at high risk of viraemia and PVN. Serum PCR testing was only carried out in patients with persistent viruria, after two positive urines. The results were only available 2–3 weeks later, up to 8 weeks after the first positive urine sample. By this time, patients with PVN had declared themselves by a rising serum creatinine in most cases, resulting in a renal biopsy. Thus, the PCR result did not play any part in management decisions. Most patients with significant positive urine cytology were treated based on the urine result, in part due to the delay in obtaining the PCR result and a lack of an agreed protocol for management of PV-positive patients. Most of these patients had stable creatinine levels at the time of treatment.

In addition to onset of viruria, clinical PVN occurred early, with all three cases diagnosed in the third month post-transplantation. This contrasts with a previous study⁸ where PVN developed at a median of 28 weeks post-transplantation and also with an earlier study in Oxford which found that the median time from transplantation to diagnosis of PVN was 6.5 months (range 3–18 months) (unpublished data). During that study, between 2000 and 2003, no patient received antibody induction and the incidence of PVN was 5%. It is possible that the current use of antibody induction accounts for the difference. In other studies, with no apparent screening, the histological diagnosis of PVN was made at a mean of 9–14.4 months post-transplantation.^{10–13} The impact of different immunosuppressive agents on the frequency and timing of PVN means that the optimum screening protocol for polyoma virus infection will vary between centres and should be reviewed with each modification of immunosuppressive regimen.

Histological PVN was diagnosed 1–6 weeks following the first significant positive urine sample. This interval is shorter than that found in another study, where viruria preceded PVN by a median of 12 weeks.⁸ Both the early onset of PVN and this short pre-clinical phase of re-activation mean that once viruria is identified, further investigation and intervention must be rapid if clinical PVN is to be avoided.

In the light of our experience, it is apparent that the original screening protocol is of limited clinical value. The positive predictive value of significant viruria for the development of clinical PVN is low; it is higher for viraemia but the results of the plasma PCR were received too late to be acted on. The protocol that we would now recommend (fig 3) is very different from current guidelines. It includes earlier, more frequent urine screening, earlier quantitation of viral load in the blood and a protocol for intervention in viraemic patients. This is by reduced immunosuppression: we found no evidence of a benefit with ciprofloxacin and there is little evidence that other anti-viral agents are effective. Currently, we do not recommend renal biopsy in the presence of good, stable graft function, even for viraemic patients, as early PVN is focal and there is a high risk of a false negative biopsy. However, our threshold for performing a biopsy is low in the presence of sub-optimal graft function in these patients. Reduced immunosuppression does carry a low but definite risk of acute rejection. Two of our patients suffered this complication and early biopsy is essential to differentiate this from PVN.

ACKNOWLEDGEMENTS

We thank Dr W Tong for performing the quantitative PCR analysis for BK virus.

Take-home messages

- Urine cytology screening for virally infected cells (decoy cells) demonstrates that urinary re-activation of polyoma virus (PV) is common following renal transplantation.
- Viruria usually occurs within the first 3 months post-transplantation and is associated with worse graft function at 3 and 6 months.
- The time between viruria and clinical PV nephropathy is short.
- If urine screening is to reduce PV nephropathy and achieve a clinical benefit, there must be frequent early screening, immediate investigation of patients who have a significant positive urine with plasma PCR, and reduction of immunosuppression if PCR is positive.

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Competing interests: None declared.

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