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Original Article

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Risk factors for polyoma virus nephropathy

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Abstract

Background. Polyoma virus-associated nephropathy (PVN) is a common cause of renal transplant failure. The risk factors for the development of PVN have not yet been studied in large cohorts of patients for periods of 20 years.
Methods. We collected clinical, renal biopsy and urinary cytology data from all patients with renal transplantations performed at the University Hospital of Basel from 1985 to 2005.

All patients with a renal biopsy and urine cytology were included ($n = 880$). Renal transplants were divided into three groups, according to evidence of polyoma virus (PV) infection (decoy cells in the urine) and biopsy-proven PVN:

1. Renal transplants without evidence of a PV infection ($n = 751$).
2. Renal transplants with PV reactivation, e.g. decoy cell (DC) found by urinary cytology, but without PVN ($n = 90$).
3. Renal transplants with PVN ($n = 39$).

Results. The prevalence of biopsy-proven PVN in this cohort of patients was 3.3%. Immunosuppression with mycophenolate and/or tacrolimus, ATGAM, male gender of the recipient and a higher number of transplant rejection episodes were factors significantly associated with PVN development.
Conclusions. The most important risk factors for the development of PVN are acute rejection and ATGAM used as induction therapy as well as tacrolimus and mycophenolate as maintenance therapy. Therefore, we conclude that patients with tacrolimus and mycophenolate maintenance therapy should be carefully monitored for the development of PVN.

Keywords: BK virus; decoy cell; polyoma virus-associated nephropathy; renal transplantation; risk factors

Introduction

Before 1971, polyoma viruses were considered to be 'viruses looking for a disease', till Gardner detected BK-virus in the urine of a patient with a renal transplant and suffering from ureteral stenosis [1]. This finding could be subsequently verified in 3 of 51 patients with ureteral complications [2].

For many years the only documented case of renal infection with BK-virus was that of a child with congenital immune deficiency syndrome [3]. It has also been known for a long time that PV can induce haemorrhagic cystitis in bone marrow transplanted patients [4–8].

In 1971, JC-virus was identified as the cause of multifocal leukoencephalopathy, which is usually fatal [9].

In 1995, BK-virus again became the focus of attention as the cause of polyoma virus nephropathy (PVN) in renal transplant recipients [10]. There have been many subsequent publications on PVN [11–53], fuelling an ongoing discussion concerning the causes of the outbreak of PVN; in particular, the risk factors for acquiring PVN are controversial. PVN can only be reliably diagnosed from a renal biopsy [11,14,54]. Viral reactivation can be diagnosed by PCR testing in blood and urine in conjunction with decoy cell (DC) detection.

The goal of this study was to identify risk factors for PVN in a series of nearly 900 renal transplant recipients. They were transplanted between 1985 and 2005, their urine was screened for DCs and more than 3000 renal biopsies, performed during periods of functional disturbances, were available. They all had a minimum of a 1-year follow-up to exclude later development of PVN. The results showed that, of all the risk factors studied, medication with tacrolimus and/or mycophenolate mofetil was the most important factor where intervention was possible.

Patients, materials and methods

Patients

During the period from January 1985 to January 2005, 1173 renal transplants were done in 1077 patients at the

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University Hospital Basel. Patients in whom no renal biopsy or urine cytology was available were excluded ($n = 293$). None of the excluded patients had PVN. Of the 293 patients, 178 had no urine samples for cytology; the remaining 115 had no biopsies. Of the latter group, 8 nephrectomy specimens and 12 autopsies were available for study, which showed no signs of PVN. The statistical comparison of all clinically relevant data showed no significant differences except for the following: shorter warm and cold ischaemia times but higher panel reactive antibody (PRA) titres and shorter survival rates for the excluded patients. All particulars refer to the numbers of transplants and not the numbers of patients, since some patients received more than one transplant ($n = 56$). In total, 880 renal transplants could be evaluated.

The following data were gathered from all patients retrospectively: sex, age, underlying disease, age at transplantation, age at death, type of donor (post-mortem or living donor, degree of family relationship, sex of donor), HLA mismatch, HLA typing of donor and recipient, PRA titre (PRA), warm and cold ischaemia time and immune suppression, both initial and during follow-up. The biopsy findings at the time of transplantation—zero biopsy—were also evaluated. The minimum follow-up period was 1 year (till 1 January 2006). The protocol for immune-suppression at the time of PVN was studied in a subset of patients.

Materials and methods

Urinary testing was done at varying intervals, usually as part of the annual control, or for early detection of urothelial neoplasia in transplanted patients with a known history of analgetic abuse and later as screening for PVN. Disturbance of renal function alone was not an indication for urinary testing.

A sample of 100 ml of freshly collected second morning urine was centrifuged and either smears (prior to 2003) or cytospin preparations were stained according to Papanicolaou. A total of 8104 urine samples were investigated. In all cases, special attention was paid to the presence of DCs, which were quantified when present. A semi-quantitative classification was used: none, few (1–4 DC/10HPF) and many (>4 DC/10HPF).

The indication for a renal biopsy was nearly always a disturbance of renal function. A total of 3009 renal biopsies (without time-zero biopsies) were available. The biopsies were studied according to standard protocols by light microscopy, immune-histochemistry and in part by electron microscopy [12].

Starting in 1998, a systematic prospective immune histochemical search of all renal biopsies for SV 40-T-Antigen (Oncogene, Research Products, San Diego, CA, USA) as evidence for PVN has been performed. In addition, to avoid any bias, we performed all biopsies before 1998 in all patients with DCs in their urine at any time after transplantation retrospectively immunohistochemistry for SV-40 antigen, cytomegalovirus, Epstein-Barr virus, Herpes simplex virus I and II and adenovirus antigens.

Based on the results of urinary testing for DC and analysis of the renal biopsy, three groups could be formed (Table 1 and for details see supplementary table S1): group 1: no DC no PVN; group 2: few (<4/10HPF) DC, no PVN; group 3: many DC (>4/10HPF); group 3.1 without PVN; group 3.2 with PVN.

Groups 1 and 2 have been combined in the results section, since there were no significant differences between these groups.

Selection of subgroups at the time-point of biopsy

Since PVN manifested at different time-points after transplantation, sub-collectives were formed (designated by S). The procedure selected was as follows. For each case in group 3.2 ($n = 39$) the closest comparable case (matched control) from groups 1–3.1 was selected, taking into account the year of transplantation, age, sex and interval between transplantation and time of peak DC excretion. This produced three subcollectives that did not differ significantly ($P > 0.05$) according to all the parameters listed in supplementary table S1 and the parameters for rejection included in supplementary table S2.

Statistical analysis

All statistical analyses were performed with the aid of the JMP 5.1 software (SAS Institute Inc., Cary, NC, USA). The chi-square test and the Mann–Whitney *U*-test were applied to compare categorical and continuous variables when appropriate. Odds ratios for the risk of developing PVN were calculated from two-by-two contingency tables (Fisher's exact test). Multivariate logistic regression analyses were used to identify risk factors for developing a PVN. The model included only those factors that were found to be significantly predictive in preceding univariate analyses. The analysis was performed with stepwise forward logistic regression (the signicance level for removing the variable from analysis 0.1, for entering the variables 0.05).

A result was only considered to be significant when the *P*-value was <0.01; *P*-values between 0.05 and 0.01 were classified as 'trends to significance'.

Results

Patient collective: demographic and transplantation-specific characteristics

The collective of 880 patients, with both renal biopsies and urine analyses, included 519 males (59%) and 361 females (41%) of mean age 48 years at transplantation and mean follow-up time of 60 months. The prevalence of PVN in this collective was 3.3%

There were 766 first transplants (87%) and 100 second transplants (11%); the remainder had three or more transplants (for details see supplementary table S1).

Of the patients, 551 received cadaver kidneys (63%) and 329 received live donor kidneys (37%); of the latter 1/3 was donor related and 2/3 donor unrelated. Of the cases, 44% were sex matched, 33% were female kidney to male recipient and 23% vice versa. The mean HLA-mismatch was 4.6, median 5.0. Only 10% had two or less mismatches, but more than 60% had five to six mismatches. The mean

Table 1. Renal biopsies in transplanted patients

	All patients with urine tests and renal biopsies	Group 1 (DC 0, PVN negative)	Group 2 (DC 1–4, PVN negative)	Group 3 (DC >4, PVN negative/positive)	Group 3.1 (DC >4, PVN negative)	Group 3.2 (DC >4, PVN positive)
No. of renal grafts (*n*)	880	595	156	129	90	39
No. of urine examinations (*n*)	8104	3922	2060	2122	1275	847
No. of urine examinations per graft (*n*)	9.3 ± 9.7 (8.0)*	6.7 ± 5.6 (6.0)	13.2 ± 12.7 (10.0)	16.4 ± 14.1 (11.0)	14.2 ± 12.1 (10.0)	21.7 ± 17.0 (12.0)
Interval transplantation—urine examination in days	860 ± 1275 (355)	923 ± 1354 (357)	984 ± 1369 (371)	623 ± 958 (296)	530 ± 652 (242)	763 ± 1276 (340)
No. of renal biopsies (*n*)	3009	2011	527	471	284	187
No. of renal biopsies per graft (*n*)	3.4 ± 1.7 (3.0)	3.4 ± 1.7 (3.0)	3.4 ± 1.6 (3.0)	3.6 ± 1.7 (3.0)	3.1 ± 1.5 (3.0)	4.8 ± 1.8 (5.0)
Interval transplantation—renal biopsies (without time-zero biopsies)	656 ± 1084 (173)	698 ± 1116 (175)	759 ± 12 667 (176)	378 ± 596 (155)	457 ± 712 (147)	266 ± 350 (157)
	1–6774	1–6360	1–6774	2–3509	2–3509	5–2269
Biopsies taken at transplantation *n* (%)	521 (17)	374 (19)	89 (17)	58 (12)	43 (15)	15 (8)

*Mean, SD (median).
DC = decoy cells, PVN = polyomavirus-associated nephropathy.

PRA-titre was low at 4.2%. The mean cold ischaemia time was 666 min; the mean warm ischaemia time was 2.7 min.

Patient groups 1 (no DC) and 2 (few, 1–4 DC) showed no difference by univariate analysis and were therefore combined (see supplementary table S1). A comparison between groups 1 and 2 combined and group 3 (many DC) revealed that DC were more frequent in men as well as in recipients of non-related grafts. When the comparison was restricted to group 3.2, it could be seen that kidneys from living donors, particularly non-related, were risk factors for PVN. Comparison between sub-groups 3.1 and 3.2 produced a similar result.

In 23 cadaver kidney transplants, both kidneys from a single donor were transplanted in Basel. Six of these transplant recipients from three kidney donors had similar findings: many DC in urine (group 3). Forty recipients receiving single kidneys from the other 20 kidney pairs were assigned to different groups in respect of DC excretion (groups 1–3.2). The only significant risk factor found was the sex of the recipient (male) ($P = 0.001$); all other parameters studied were not relevant. Due to the contemporaneous transplantation of the kidney pairs, practically all received the same therapy, so that no statement concerning therapy modality as a risk factor is possible.

In conclusion, an increased risk of strong reactivation of a polyoma virus infection with/without PVN was seen in male patients and in recipients of living donor kidneys, especially those from non-related donors. All other parameters did not have a significant influence on reactivation of polyoma virus with excretion of many DC, with/without PVN.

Immune suppression

Initial immune suppression. Immune suppression protocols were subject to many changes over the years concerned. This is reflected in supplementary table S3, where the immune suppressives employed at the time of transplantation are summarized.

Induction therapy was usually done with antithymocyte antigens (ATG)/antilymphocyte antigens (ALG) ($n = 302$) or IL-2 receptor-directed antibodies (basiliximab, daclizumab) ($n = 133$); 370 patients received no induction therapy.

The most common basis immune-suppressive drug used was cyclosporin (CyA) ($n = 662$), followed by tacrolimus (Tac) ($n = 138$) and rapamycin (Rapa) ($n = 65$). Co-medication was usually done with azathioprine (Aza) ($n = 563$) and less frequently with mycophenolate mofetil (MMF) ($n = 296$). Co-medication with steroids was done in practically all cases and therefore steroid therapy was not taken into further consideration.

Therapy with tacrolimus and MMF (Table 2) was associated with a risk for many DC in urine (group 3). ATGAM, IL-2 receptor-directed antibodies (basiliximab, daclizumab) and MMF were risk factors for PVN (groups 1 and 2 versus 3.2).

A total of 11 different therapy schemes were defined, taking into account the induction therapy, basis immune suppression and co-medication (excluding steroids) (supplementary table S4). In 39 patients combinations of medicaments were used that <1% of all patients received; these were not considered further. The most frequent therapy schemes were ALG-CyA-Aza ($n = 276$), CyA-MMF ($n = 125$) and CyA monotherapy ($n = 115$).

In respect of induction therapy (groups 1 and 2 versus 3 or 3.1), only the use of ATGAM was associated with a clearly increased risk of polyoma virus reactivation. The risk of reactivation was increased significantly with tacrolimus as basis immune suppression and MMF a co-medication (Table 3). The appearance of PVN itself was associated with ATGAM and/or tacrolimus therapy.

The importance of tacrolimus and/or MMF can be seen when the relative frequencies of these therapy forms in

Table 2. Relation between DC excretion and individual substances

Initial immunosuppressive therapy	Groups 1 and 2 versus group 3		Groups 1 and 2 versus group 3.1		Groups 1 and 2 versus group 3.2		Group 3.1 versus group 3.2	
	P	OR	*P*	OR	*P*	OR	*P*	OR
Induction therapy								
ATG versus no ATG	**<0.0001**	**0.3**	**0.0015**	**0.4**	**<0.0001**	**0.1**	**0.0138**	**0.2**
ATGAM versus no ATGAM	**0.0377**	**2.3**	0.6456	1.3	**0.0037**	**5.1**	**0.042**	**3.9**
OKT-3 versus no OKT-3	0.1737	0.5	0.5153	0.7	0.0533	0.1	0.1388	0.1
IL-2 receptor directed antibodies versus no	0.0942	1.5	0.8127	0.9	**0.0011**	**3.4**	**0.0045**	**3.6**
Induction therapy versus no induction	**0.0046**	**0.6**	**0.0014**	**0.5**	0.6642	0.9	0.1379	1.8
ATG versus no induction	**<0.0001**	**0.3**	**0.0004**	**0.4**	**0.0005**	**0.1**	0.1124	0.3
ATGAM versus no induction	0.2050	1.7	0.8334	0.9	**0.0132**	**4.1**	**0.0286**	**4.6**
OKT3 versus no induction	0.0619	0.4	0.198	0.6	0.0531	0.1	0.1982	0.1
IL-2 receptor directed antibodies versus no induction	0.8205	1.1	0.1881	0.6	**0.0296**	**2.3**	**0.0079**	**3.6**
Basis immune suppression								
CyA versus no	**<0.0001**	**0.4**	**0.0009**	**0.4**	**0.0109**	**0.4**	0.8199	0.9
Tacrolimus versus no	**<0.0001**	**2.5**	**0.0004**	**2.6**	0.0526	2.2	0.7042	0.8
Rapamycin versus no	0.5986	1.2	0.8539	0.9	0.2306	1.9	0.2659	2.1
Co-medication								
Aza versus no	**0.0001**	**0.5**	**0.0177**	**0.6**	**0.0041**	**0.4**	0.4536	0.8
MMF versus no	**0.0001**	**21.5**	**0.0035**	**1.9**	**0.0041**	**2.6**	0.4536	1.3

ATG = antithymocyte globulin, ALG = antilymphocyte globulin, IL-2-RA = IL-2 receptor-directed antibodies (basiliximab and daclizumab), CyA = cyclosporin-A, Tac = tacrolimus, Rapa = rapamycin, Aza = azathioprine, MMF = mycophenolate mofetil, DC = decoy cells, PVN = polyomavirus-associated nephropathy, OR = odds ratio.

Table 3. Relation between the presence of DC in urine and various combination therapies

Initial immunosuppressive therapy	Groups 1 and 2 versus group 3		Groups 1 and 2 versus group 3.1		Groups 1 and 2 versus group 3.2		Group 3.1 versus group 3.2	
	P	OR	*P*	OR	*P*	OR	*P*	OR
CyA-Aza (without induction) compared with								
Alg/ATG-CyA-Aza	0.1226	2.5	**0.0137**	**6.9**	0.1955	0.2	**0.0192**	**0.1**
ATGAM-CyA-Aza	**<0.0001**	**15.0**	**0.0034**	**17.9**	**0.0008**	**13.4**	0.8340	0.8
OKT3-CyA-Aza	0.1855	3.1	**0.0360**	**9.3**	0.2929	0.1	0.0507	0.1
Tac-Aza (without induction) compared with								
IL-2-RA-Tac-Aza	0.1425	0.3	**0.0132**	**0.1**	0.8376	0.8	0.1425	6.3
CyA-MMF (without induction) compared with								
Alg/ATG-CyA-MMF	0.5527	0.6	0.3340	0.4	0.6979	1.6	0.3641	4.0
IL-2-RA-CyA-MMF	0.7678	0.9	0.1588	0.5	0.1334	2.4	**0.0296**	**4.7**
Rapa-MMF (without induction) compared with								
IL-2-RA-Rapa-MMF	0.4718	1.6	0.9314	0.9	0.2091	3.7	0.2945	4.0
CyA-Aza (without induction) compared with								
Tac-Aza (without induction)	**<0.0001**	**17.5**	**<0.0001**	**39.8**	**0.0118**	**6.3**	0.1425	0.2
CyA-MMF (without induction)	**<0.0001**	**11.8**	**<0.0001**	**28.3**	0.1001	3.5	0.0996	0.1
Rapa-MMF (without induction) compared with								
CyA-MMF	0.1849	0.5	0.1950	0.5	0.6353	0.6	0.8200	1.3

ATG = antithymocyte globulin, ALG = antilymphocyte globulin, IL-2-RA = IL-2 receptor-directed antibodies (basiliximab and daclizumab), CyA = cyclosporin-A, Tac = tacrolimus, Rapa = rapamycin, Aza = azathioprine, MMF = mycophenolate mofetil, DC = decoy cells.

groups 1 and 2, as well as 3.1 and 3.2, are considered (supplementary table S4). In groups 1 and 2 only 42% of the patients received one or both substances, compared to 73% in group 3.1 and 77% in group 3.2.

In summary, the findings speak for an increased risk of viral reactivation with/without PVN with the use of ATGAM as induction therapy, with tacrolimus as basis immune suppression and with MMF co-medication. All other therapy schemes were only associated with low risks.

Immune suppressive maintenance therapy. To help judge the value of the findings concerning the importance of the initial immune suppression for the reactivation of polyoma virus, the immune suppressive maintenance therapy was studied in a subgroup of groups 1 and 2-S, as well as 3.1-S and 3.2-S.

In our cases, PVN was diagnosed after a mean period of 283 ± 232 days (median: 196, range 42–982). Thus, the initial immune suppression can only give limited information

Table 4. Relation between DC excretion and PVN with different treatment combinations in subgroups

Therapy combinations	Group 1/2-S versus group 3-S		Group 1/2-S versus group 3.1-S		Group 1/2-S versus group 3.2		Group 3.1-S versus group 3.2	
	P	OR	*P*	OR	*P*	OR	*P*	OR
Rapa-(CyA)-MMF versus CyA-Aza/CyA only	0.1819[a]	4.7	0.3022[a]	3.5	0.2264	1.2	0.4722[a]	0.3
Rapa-(CyA)-MMF versus CyA-MMF	0.1907	0.4	0.4198	0.5	0.1384	4.5	0.4500	2.4
Rapa-(CyA)-MMF versus Tac-MMF	0.0632	1.5	1.000[a]	1.0	**0.0086[a]**	**21.0**	**0.0293[a]**	**21.0**
CyA-Aza/CyA only versus CyA-MMF	**0.0065**	**11.8**	0.0503	6.5	**0.0093**	**5.3**	0.6140	1.6
CyA-Aza/CyA only versus Tac-Aza	**<0.0001**	**47.3**	**0.0026**	**21.0**	**<0.0001**	**26.3**	0.2091	2.5
CyA-MMF versus Tac-MMF	**0.0028**	**28.0**	0.6140	0.5	0.0618	4.7	**0.0234**	**8.8**
Tac-Aza versus Tac-MMF	0.5917	0.6	0.1698[a]	0.2	0.9440	0.9	0.0805	5.6

[a]Small numbers.
ATG = antithymocyte globulin, ALG = antilymphocyte globulin, IL-2-RA = IL-2 receptor-directed antibodies (basiliximab and daclizumab), CyA = cyclosporin-A, Tac = tacrolimus, Rapa = rapamycin, Aza = azathioprine, MMF = mycophenolate mofetil, DC = decoy cells, PVN = polyomavirus-associated nephropathy.

about the relationship between immune suppression at the time of biopsy and the extent of polyoma virus reactivation. Complications following rejection crises, drug intolerance, etc. frequently necessitated changes in the type of immune suppression. Groups 1 and 2-S on one hand and 3.1-S and 3.2-S on the other hand differ only in respect of therapy; there were no significant differences ($P > 0.1$) in any of the other parameters studied, including renal biopsy findings.

Analysis of the individual immune suppressive agents employed showed that of all maintenance therapies used, tacrolimus (but not MMF) is associated with an increased risk of pronounced viral reactivation with/without PVN (the significance for MMF was not seen in our small subgroup analysis). In addition, bolus therapy with steroids is also linked to an increased risk for PVN (see supplementary table 5S).

The therapy schemes most frequently employed included tacrolimus and/or MMF. In group 1/2-S, 77% received tacrolimus and/or MMF; in group 3.1-S, 95% and in group 3.2, 100% (supplementary table S6). Of the treatments used (Table 4), CyA-MMF, Tac-Aza and Tac-MMF appear to be particularly high-risk combinations.

Analysis of serum drug concentrations (see supplementary table S7) revealed a borderline significance between tacrolimus levels and a marked reactivation of polyoma virus, with/without PVN. In group 1/2-S, the median level of tacrolimus was 11.5 and in groups 3.1-S and 3.2-S, 17.9 and 18.0 ng/ml, respectively.

For CyA levels between 300 and 500 μg/l (median), rapamycin levels between 15 and 20 μg/l (median), MMF levels between 4 and 5 mg/l (median) and total steroid dosage between 2 and 3 g (median), there were no significant differences ($P > 0.01$) between the groups in respect of viral reactivation/PVN.

In summary, analysis of maintenance immune suppression revealed an association between tacrolimus and/or MMF and PV reactivation with/without PVN.

Renal biopsy findings in relation to DC excretion. The most common findings were interstitial rejection or calcineurine inhibitor toxicity, each in ∼50% of the transplants.

Patients in group 3, which is with strong reactivation of polyoma virus with/without PVN, had interstitial rejection significantly more frequently and revealed signs of a humoral rejection reaction with transplant glomerulitis (see supplementary table S2).

A time-zero biopsy at the time of transplantation was available in 58% of the patients ($n = 511$). Pre-existing damage to the donor kidney had no influence on DC excretion.

Comparison of the sub-groups 3.1 or 3.2 (many DC, without/with PVN) with the group without/few DC revealed a trend towards more vascular and interstitial rejection in group 3.1, but a highly significant number of cases of humoral rejection in group 3.2 (signalled by C4d positivity in peritubular capillaries) sometimes with and sometimes without transplant glomerulitis (see supplementary table S2). Also in patients with many DC in urine (group 3), pre-existing damage to the donor kidney had no influence on the subsequent development of PVN.

In summary, it can be stated that rejection crises of all types are associated with PVN, particularly in group 3.2, and very frequently exhibit characteristics of humoral rejection (transplant glomerulitis, C4d positivity in peritubular capillaries). Of interest, calcineurine inhibitor nephrotoxicity and pre-existing renal damage had no obvious relevance.

Summary of risk factors: correlation of risk factors with each other and results of multi-variate analysis. The results of the multivariate Cox regression analysis of possible factors influencing PV reactivation as well as manifestation of PVN are summarized in Table 5. The results show no differences when all parameters, or only those parameters significant in the univariate analysis, are considered. The most important factors that favour a reactivation of the PV infection and/or manifestation of PVN are sex of recipient (male), non-related live donor, tacrolimus, MMF and ATGAM treatment, and rejection reactions (rejection score: interstitial, vascular rejection, transplant glomerulitis and C4d positivity in peritubular capillaries). Manifestation of PVN (no PVN versus PVN) is also favoured by the same factors, with the exception of sex of recipient and the parameter: non-related donor.

Table 5. Multivariate Cox regression analyses

Risk factor	Group 3 versus groups 1 and 2 ($n = 880$)		PVN (group 3.2) versus no PVN (groups 1, 2 and 3.1) ($n = 880$)		PVN (group 3.2) versus DC high (group 3.1) ($n = 129$)	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Male recipient	2.2 (1.5–3.3)	0.0001	1.5 (0.7–3.1)	0.2621	0.7 (0.3–1.5)	0.3418
Unrelated living donor	1.9 (1.1–3.4)	0.0271	2.0 (0.8–5.4)	0.1442	1.2 (0.4–3.2)	0.7249
ATGAM	2.9 (1.4–5.7)	0.0027	6.6 (2.3–18.9)	0.0005	2.9 (0.8–10.5)	0.1106
Tacrolimus	4.8 (3.1–7.3)	0.0000	3.3 (1.5–7.6)	0.0038	1.4 (0.5–3.9)	0.4715
MMF	4.6 (3.1–6.7)	0.0000	3.5 (1.6–7.5)	0.0013	2.2 (0.8–5.6)	0.1046
Acute rejection	1.9 (1.3–2.9)	0.0010	5.1 (1.8–14.6)	0.0021	3.1 (1.1–9.2)	0.0368

MMF = mycophenolate mofetil, DC = decoy cells, PVN = polyomavirus-associated nephropathy.

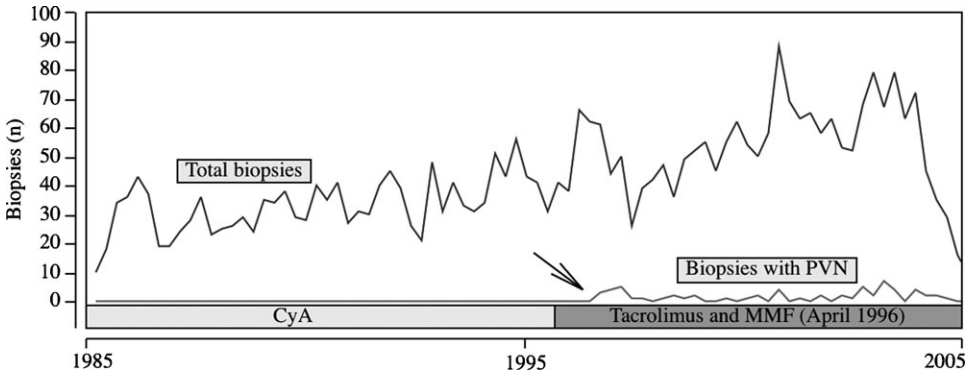

Fig. 1. Biopsies with/without PVN in relation to therapy in a 20 year period in Basel CyA = cyclosporin-A, MMF = mycophenolate mofetil, PVN = polyomavirus-associated nephropathy.

The odds ratios for therapy-associated factors were higher than transplant-specific factors, which may indicate greater importance.

Discussion

This is a retrospective analysis of all renal transplantations performed in Basel between 1985 and 2005 in which urine samples (>8000) were studied for DCs and biopsies (>3000) were performed in case of renal dysfunction. To avoid any bias, all biopsies of patients having DCs in their urine were retrospectively stained for SV40 to prove or exclude BK-virus.

Primarily, all patients, finally excluded ($n = 293$), with urine samples but no biopsies or biopsies but no urine samples for cytology were included in the evaluation without any difference to the results described in this paper. Of note, no patients with PVN were excluded from the analysis.

The prevalence of PVN in this series was 3.3%, which is in the range reported by other investigators (1.1–9.3%) [23,24,30,32,36,55–62]. Our study covered the highest number of potential risk factors studied to date. Of note, multivariate analysis revealed that only three groups of factors were significantly associated with the reactivation of a polyoma virus infection and particularly PVN: immune suppression, rejection and male gender of transplant recipients and non-related live donor kidneys.

Regarding immune suppression after transplantation, all commonly used substances and combinations thereof were evaluated. Here, it appeared that only three immune suppressives favoured development of PVN: tacrolimus, MMF and the now uncommon ATGAM. All other immune suppressives in common use, particularly cyclosporin, azathioprine, rapamycin, ALG and specific anti-lymphocyte preparations had no obvious influence on the development of PVN. During application of the latter substances, PVN was not observed, although there was some reactivation of PV. This result was obtained from analysis of all patients, taking the initial immune suppression into account, as well as from analysis of a sub-collective in which the maintenance therapy was followed up for a mean of 9.4 months (median 7.7) after transplantation. Only in the case of tacrolimus, not for the other immune suppressives, did rising blood levels increase the risk of strong PV reactivation with/without PVN. The results of previous publications on therapy regimes as particular risk factors for PVN have been conflicting.

Depending on the study, 50–100% of patients with PVN had received tacrolimus and 40–97% MMF [15,21–24,30,32,43,55–61,63–68]. This encouraged many authors to regard tacrolimus or MMF as risk factors [12,13,20,54,55,69,70]. PVN develops only rarely in patients receiving neither tacrolimus nor MMF (39 patients [2,24,35,36,55,56,61,63]). This includes a report from India [36], in which a high frequency of PVN (30 cases, prevalence 10%) was found in patients not receiving tacrolimus or MMF. A further study describes six patients with PVN not given tacrolimus, but no information regarding comedication with MMF was given [66]. Others found that tacrolimus, but not MMF, was a risk factor [67]. Two large studies from the USA with over 1000 kidney recipients did not identify any particular form of immune suppression,

including tacrolimus and MMF, as risk factors [36,47,66]. These contradictory results can be explained by the fact that the time periods covered were short and particular immune suppressives were favoured during this period. In our patients 662/880 received CyA, 138/880 tacrolimus and 65/880 rapamycin as basis immune suppression; 296/880 received MMF in addition. It is noteworthy that only 9/880 patients without either tacrolimus or MMF initially developed PVN. Of these nine patients, seven later received tacrolimus and MMF, one only tacrolimus and one only MMF. In the whole series of kidney transplant recipients, 50% received tacrolimus, 40% cyclosporine and 7% rapamycin. If immune suppressive therapy is irrelevant for the development of PVN, a much higher number of cases of PVN with cyclosporin and rapamycin therapy could be expected. Incidentally, the first case of PVN in Basel was diagnosed on 1 October 1997, approximately 1 year after the introduction of tacrolimus (April 1996, see Fig. 1). This strongly suggests that tacrolimus and MMF, but not other frequently used immune suppressive agents, increase the risk of polyoma virus reactivation and subsequent PVN [45]. The risk is further increased when tacrolimus, MMF with prednisone and additional anti-lymphocyte preparations are combined. This proves that PVN only manifests under immune suppression. Although there was a temporal coincidence between the introduction of the new, potent immune suppressives tacrolimus and MMF and the appearance of PVN, it can be assumed that the higher intensity of immune suppression with tacrolimus and MMF was decisive, rather than an additional effect of a particular class of substances [13,14,25,47,71,72]. This assumption remains controversial [61] but is supported by the association between dosage and/or blood level and risk of PVN [73]. A tacrolimus level exceeding 8 ng/ml [38,55] or a MMF-dose of over 2 g/day were associated with an increased risk for PVN [55].

The second important risk factor identified in this study was a rejection episode. By multivariate analysis, interstitial cellular rejection and transplant glomerulitis were particularly important. However, due to the odds ratio, their importance must be placed lower than the therapy. This statement must be generalized to be meaningful; it does not mean that other forms of rejection are not associated with a higher risk, rather that severe rejection episodes, regardless of type, increase the risk of PVN, as shown by the univariate analysis (see Table 5). This suggests that a severe rejection crisis is a therapy-independent factor that promotes the development of PVN. It can also be postulated that increased tubular epithelial regeneration, following tubular necrosis caused by rejection, contributes to the reactivation of polyoma virus infection, a viewpoint also previously favoured by the authors [13,17].

Interestingly acute tubular necrosis caused by both cold and warm ischaemia is also associated with accelerated regeneration of the tubular epithelium but does not favour the development of PVN, since then PVN should develop early after transplantation and not many months later. Also, severe, recurring episodes of rejection often necessitated the use of high dose tacrolimus and MMF as so-called rescue therapy. In line with this observation is the fact that patients with strong reactivation, with/without PVN, received more steroid boluses and hence a higher cumulative dose of steroids. Other authors also reported that many patients had received therapy for rejection episodes before the diagnosis of PVN [14,21,23,55]. This is particularly impressive in patients given ATGAM initially, as shown in our study to be a risk factor. Unpublished results from Basel showed that patients given ATGAM suffered much more frequently from severe rejection crises than patients given ATG, which made a change to tacrolimus therapy necessary. Interestingly, calcineurine inhibitor nephrotoxicity did not correlate with PVN. In calcineurine inhibitor nephrotoxicity (CIN) tubular necroses are also seen, without any significant increase in PVN. Taken together, tubular cell necrosis *per se* is unlikely to play a role as a cofactor in polyoma virus infection. According to the opinion of earlier investigators [21,55], including ourselves, acute rejection is an independent risk factor, although other studies couldn't demonstrate a direct association between rejection and PVN [14,15,19,24,30,32,38,43,45,61,65,66,73,74].

Multivariate analysis revealed that male gender of transplant recipients is an independent risk factor, which concurs with previous reports [24,38,56]. However, several other studies did not reveal a preference for males [14,15,30,47,55,61,66]. These differences might be explicable by differences between the groups studied. In our series rank-correlation analysis revealed a close, complex relationship between male recipients, living donor kidneys and non-related live donors.

Such complex correlations might explain why a further study found that kidneys from female donors were an independent risk factor [66], which was neither confirmed in another study [73], nor by us. The same holds true for HLA mismatches. In three studies, the extent of HLA-mismatch was reported to be an independent risk factor [23,43,55] while the majority of studies, including our own, speak against an association between HLA-mismatch and PVN [24,46,55,61,73]. CIN did not play any role in our collective, in contrast to other reports [43,56]. Diabetes mellitus

Table 6. Summary of risk factors mentioned in the literature

Risk factors	Significant risk factor	Not a risk factor
Patient-specific risk factors		
Recipient older than 50 years	[1–3]	[4–7]
White ethnicity	[3,8,9]	[4,5]
Male recipient	[2,3,9]	[1,4–7,10–16]
Previous rejections	[6,17–20]	[1,2,4,5,7,9–12,14, 15,21–25]
Ureteral stents	[23]	
BKV serostatus	[10,15,26,27]	
Disturbances of interferon γ-metabolism	[28–30]	
Diabetes mellitus	[3,11,31,32]	[1,2,4, 7]
CMV infection	[19,21,33]	[7,25]
Graft-specific risk factors		
HLA mismatch	[5,6,17]	[1,2,4,6,14,34]
Tacrolimus toxicity	[3]	[5]
Female donor	[1]	[4]
Lack of HLA C7	[34]	
Virus-specific risk factors	[35,36]	

is commonly considered to promote PVN, an opinion that others and we could not confirm (see Table 6).

In summary, we conclude that immune suppression per se promotes reactivation of polyoma virus. Tacrolimus and MMF carry an increased risk for PVN. This is evidenced in our series as the relative frequency of tacrolimus and/or MMF therapy, taking the initial therapy into account, increases from 30% in group 1 (no DC) to 50% in group 2 (<4 DC/HPF) to 75% in group 3 (>4 DC/HPF). When maintenance therapy was considered, all of the patients with PVN had received tacrolimus and/or MMF. Furthermore, the tacrolimus blood level was associated with the development of PVN. A tacrolimus level exceeding 20 ng/ml appears to be associated with a particularly high risk. The MMF level could also play a role but was not apparent in this study. Thus, not a particular drug class but the higher immune suppressant potential is most likely responsible for the outbreak of PVN in recent years. A rejection episode is an independent risk factor that increases the risk of PVN probably through subsequent intense immune suppression since before the introduction of tacrolimus and/or MMF, PVN was never seen in our patients. The various recipient and donor characteristics, such as male recipients, live donor kidneys and non-related, living donors, are also associated with a higher risk to rejection, but they probably play a secondary role. Finally, we wish to point out that other factors, factors e.g. including viral status of the recipient, white ethnicity, ureteral stents, disturbances of interferon γ-metabolism and lack of HLA C7, which have been discussed in the literature but not studied by us, should not be ignored.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

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