BK virus infection in renal transplant recipients: single centre experience

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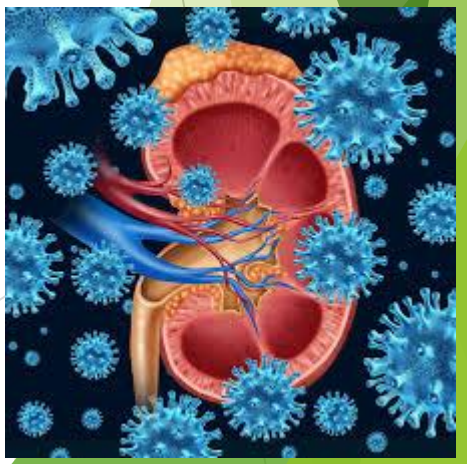
Background

- BK virus nephropathy (BKVN) has emerged as an important cause of renal graft dysfunction in recent decades
- It was first discovered in 1971 in a renal transplant patient named B.K. who developed ureteric stenosis after the viral infection
- With introduction of newer and more potent immunosuppressive agents, the incidence of BK virus nephropathy has been increasing in recent years.



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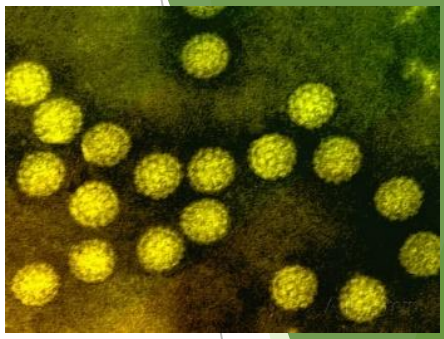


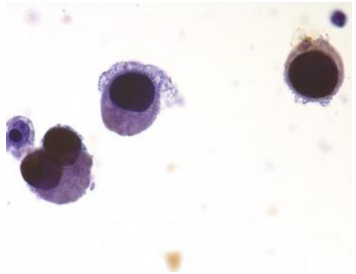
- The incidence of BK virus nephropathy is estimated at 1% to 10%
- Various risk factors for BK virus infection have been described but the **most important one is the degree of immunosuppression**
- Other potential risk factors include extremes of age, diabetes mellitus, deceased donation, more HLA mismatches, delayed graft function, treatment of acute rejection, cytomegalovirus infection and African American ethnicity

Background

- The gold standard for diagnosis of BKVN remains to be renal biopsy
- Reduction of overall immunosuppression is the mainstay of treatment for BKVN
- Evidence on other adjunctive therapies, e.g. leflunomide, cidofovir, fluoroquinolones, intravenous immunoglobulin, has been conflicting and there was no standardized treatment guidelines worldwide
- Despite various treatment approaches, graft survival is poor
  - Graft loss up to 90% was quoted in some case series

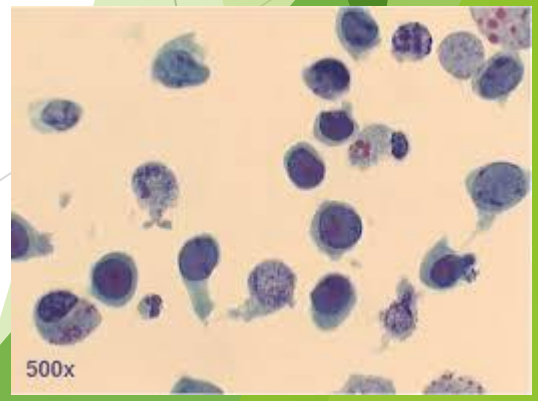
Background

- New diagnostic tools including **quantitative urine and plasma BKV DNA polymerase chain reaction (PCR) assay** allow detection of early BKV infection and disease monitoring
- It has been suggested that persistent viruria more than 10^7 viral copies/ml or viraemia more than 10^4 viral copies/ml for 3 weeks is highly correlated with BKV nephropathy



Background

- **Screening protocol for BK virus infection has been implemented in our centre since 2011**
  - Routine screening for **urine decoy cell** will be performed in new transplant recipients at 3 monthly intervals post-transplant
  - If urine decoy cell is negative, urine screening will be continued every 3 months till 2 years, followed by annually till 5 years post- transplant
  - If urine decoy cell is positive, **plasma BKV DNA PCR assay** will be done
  - If plasma BKV DNA is positive but < 10^4 viral copies/ml and there is no graft dysfunction, **preemptive treatment with reduction in immunosuppressants** will be started and plasma BKV DNA PCR will be monitored monthly
  - If plasma BKV DNA is >10^4 viral copies/ml, **renal biopsy** will be performed to document BKV nephropathy. Patients with biopsy proven BKV nephropathy will be treated by reduction of immunosuppressants. Adjunctive therapies will also be considered



Background

- Despite increasing awareness of BK virus nephropathy worldwide, there is limited study to date assessing the disease in Chinese population
- **The primary objective of this study is to assess the incidence and risk factors of BK virus nephropathy in renal transplant recipients in our locality**
- Secondary objectives include review of clinical course, management strategy and outcome of patients with BK virus nephropathy.

Methods

Study Design

- This is a **case-control study** of renal transplant recipients in Princess Margaret Hospital from **January 2000 to January 2014**
- **The study group included patients with biopsy-proven BKVN during the period**
  - These patients had graft biopsies done because of graft dysfunction or plasma BKV DNA >10^4 copies/ml
  - Patient who were followed-up for less than 12 months after diagnosis of BKVN were excluded.
- **The control group included 4:1 randomly selected patients who underwent renal transplantation during the study period and had no evidence of BK virus infection.**
  - Age and transplant year stratified simple randomization were done by computer-generated random number.
  - Patients who had positive urine decoy cell, positive plasma BKV DNA and follow-up duration less than 12months were excluded.

Risk Factors

- **Risk factors for BKVN were evaluated for donor, recipient and transplant risk variables**
  - Age, recipient gender, primary renal disease, diabetes mellitus, history of previous renal transplantation, number of human leukocyte antigen (HLA) mismatches, deceased donor status, use of induction therapy, choice of immunosuppressant, history of acute rejection, delayed graft function, cytomegalovirus infection and ureteral trauma

Management

- Patients with biopsy-proven BKVN in our centre were managed primarily by **reduction of immunosuppression**
  - By reducing or stopping the antimetabolite, i.e. mycophenolate mofetil (MMF) or azathioprine (AZA), reducing tacrolimus (FK) to aim at serum level of 3-7 ng/ml, reducing cyclosporine (CsA) to aim at serum level of 40-60 ng/ml, reducing sirolimus (SRL) or everolimus (EVL) to aim at serum level of 3-5 ng/ml, switching tacrolimus to cyclosporine, or combination of the manoeuvres.
- Other adjuvant therapies include switching antimetabolite to mammalian target of rapamycin (mTOR) inhibitor, use of leflunomide, fluoroquinolones and intravenous immunoglobulin (IVIG)

Clinical course and Outcome measures

- The clinical course and outcome of patients with BKVN were reflected by the **serum creatinine** (SCr, umol/L) and **plasma BKV DNA** (copies/ml) at different time interval during the study period
- Presence of **acute rejection after diagnosis of BKVN, graft loss and death** were also evaluated.

Histological pattern on renal biopsy

- **Banff ct score** for assessment of tubular atrophy (ct0: no tubular atrophy, ct1: up to 25% cortical tubules, ct2: 26-50%, ct3: >50%)
- **Drachenberg grade** (pattern A- early: no or insignificant inflammation, pattern B- florid: significant inflammation, pattern C- late: >50% interstitial fibrosis and tubular atrophy)
- **Polyoma viral load level** (pvl 1: <1% tubules with viral replication, pvl 2: >1% to <10% tubules with viral replication, pvl 3: >10% tubules with viral replication)

Statistical Analysis

- Statistical analysis was performed using SPSS for windows software version 22
- The demographical data and biochemical parameters were expressed as mean +/- SD, median, interquartile range [IQR, 25^{th} – 75^{th} percentile], number or percentage as appropriate
- **Identification of risk factors were done by univariate logistic regression analysis**
- The clinical course, management strategy and outcome of patients with BKVN were analysed by descriptive statistics

Results

Incidence

- From January 2000 to January 2014, a total of **15 patients** had biopsy-proven BKVN.
- During this study period, 676 renal transplants were carried out, giving an **incidence of 2.22%**.
- The control group included 60 patients without evidence of BKVN.

Demographic characteristics

- The **median age at transplant for both groups was 39 years** (IQR 27-52 years vs 28.5-57 years, p=0.745).
- Among patients with BKVN, 66.7% were male and 33.3% were female, which was not significantly different from the control group (46.7% male and 53.3% female, p=0.166).
- No significant association was observed between occurrence of BKVN and history of previous renal transplant, cold ischemic time, second warm ischemic time, type of donor, use of induction therapy, number of immunosuppressant, delayed graft function, cytomegalovirus infection and ureteral trauma.

Table 1. Demographic and clinical characteristics of subjects

	Case (n=15)	Control (n=60)	*p* -values [a]	Total (n=75)
	Count (%)	Count (%)		Count (%)
Age at transplant (year)[†]	39 [27, 52]	39 [28.5, 57]	0.745 [b]	39 [28, 56]
Male gender	10 (66.7)	28 (46.7)	0.166 [c]	38 (50.7)
Primary renal disease			–	
Glomerulonephritis	5 (33.3)	31 (51.7)		36 (48.0)
Polycystic kidney disease	2 (13.3)	0 (0.0)		2 (2.7)
Diabetic nephropathy	0 (0.0)	5 (8.3)		5 (6.7)
Congenital anomaly	1 (6.7)	7 (11.7)		8 (10.7)
Unknown	7 (46.7)	11 (18.3)		18 (24.0)
Reflux nephropathy	0 (0.0)	3 (5.0)		3 (4.0)
Hypertensive nephropathy	0 (0.0)	3 (5.0)		3 (4.0)
Diabetes mellitus	6 (40.0)	6 (10.0)	0.011	12 (16.0)
History of previous transplant	2 (13.3)	3 (5.0)	0.260	5 (6.7)
Number of HLA mismatch[†]	4 [3, 5]	3 [2,3]	0.024 [b]	3 [2,3]
Cold ischemic time (min)[†]	410 [214.25, 600]	485 [368.5, 720]	0.227 [b]	480 [360, 720]
Second warm ischemic time (min)[†]	45 [35, 55]	40 [32.25, 45]	0.128 [b]	40 [34, 46]
Type of donor			0.499	
Deceased	13 (86.7)	44 (73.3)		57 (76.0)
Living	2 (13.3)	16 (26.7)		18 (24.0)
Use of induction therapy	5 (33.3)	15 (25.0)	0.526	20 (26.7)
Type of induction therapy			–	
Nil	11 (73.3)	45 (75.0)		56 (74.7)
Antithymocyte globulin	3 (20.0)	7 (11.7)		10 (13.3)
Daclizumab	1 (6.7)	6 (10.0)		7 (9.3)
Basilixumab	0 (0.0)	1 (1.7)		1 (1.3)
Alemtuzumab	0 (0.0)	1 (1.7)		1 (1.3)
Plasmapheresis	0 (0.0)	0 (0.0)		0 (0.0)
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Anti-IL2R induction	1 (6.7)	7 (11.7)		8 (10.7)
Number of immunosuppressants			1.000	
Two	0 (0.0)	2 (3.3)		2 (2.7)
Three	15 (100.0)	58 (96.7)		73 (97.3)
Immunosuppresssant used				
Prednisolone	15 (100.0)	60 (100.0)	–	75 (100.0)
Tacrolimus	13 (86.7)	29 (48.3)	0.007 [c]	42 (56.0)
Cyclosporine	2 (13.3)	30 (50.0)	0.010 [c]	32 (42.7)
Mycophenolate mofetil	13 (86.7)	51 (85.0)	1.000	64 (85.3)
Azathioprine	0 (0.0)	5 (8.3)	0.576	5 (6.7)
Sirolimus	1 (6.7)	1 (1.7)	0.362	2 (2.7)
Everolimus	0 (0.0)	2 (3.3)	1.000	2 (2.7)
Higher level of immunosuppression (induction and/or FK)	14 (93.3)	34 (56.7)	0.008 [c]	48 (64.0)
Number of previous acute rejection[†]	0 [0, 1]	0 [0, 0]	0.057 [b]	0 [0, 1]
History of previous acute rejection	7 (46.7)	14 (23.3)	0.106	21 (28.0)
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Ureteral trauma	0 (0.0)	1 (1.7)	1.000	1 (1.3)

[a] Fisher's exact test.
[b] Mann-Whitney U test.
[c] Pearson's Chi-square test.
[†] Presented as median [quartile 1, quartile 3].
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Risk factors (1)

- More patients with BKVN were found to have coexisting **diabetes mellitus** (40% vs 10%, p=0.011)
- There was significant association between diabetes mellitus and occurrence of BKVN (odds ratio [OR] 6, p=0.008, 95% confidence interval [CI] 1.6-22.8).

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Intravenous immunoglobulin	0 (0.0)	0 (0.0)		0 (0.0)

Table 2. Association between subjects' characteristics and outcome: univariate logistic regression analysis.

	OR (95% CI)[†]	*p* -values
Male gender	2.286 (0.697, 7.493)	0.172 *
Diabetes mellitus	6.000 (1.581, 22.768)	0.008 ***
Number of HLA mismatch	2.299 (1.131, 4.675)	0.021 ***
Second warm ischemic time (min)	1.063 (0.983, 1.151)	0.127 *
FK	6.948 (1.442, 33.48)	0.160 *
CsA	0.154 (0.032, 0.741)	0.020 ***
High level of immunosuppression (induction/ FK)	10.706 (1.321, 86.732)	0.026 ***
Number of previous acute rejection	3.118 (1.051, 9.251)	0.040 ***
History of previous acute rejection	2.875 (0.886, 9.334)	0.079 **

[†] Unadjusted odds ratio derived from univariate logistic regression.

* p <0.2; ** p <0.1; *** p <0.05.

Risk factors (2)

- The median **number of HLA mismatches** for patients with BKVN was 4 (IQR 3-5), which was significantly higher than the control group (median 3, IQR 2-3, p=0.024)
- 37.3% of the data on number of HLA mismatches was missing as some patients had renal transplantation done in the private sector
- While higher number of HLA mismatches appeared to be associated with BKVN (OR 2.3, p=0.021, CI 1.1-4.7), its role as a risk factor cannot be established in view of small sample size secondary to presence of missing data.

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Risk factors (3)

- There was significantly **more patients with BKVN being on tacrolimus** (86.7% vs 48.3%, p=0.007)
- Use of tacrolimus (FK) appeared to have probable increased risk of BKVN although it was not statistically significant (OR 6.9, p= 0.16, CI 1.4-33.5)
- On the other hand, there were significantly **fewer patients with BKVN being on cyclosporine A** (13.3% vs 50%, p=0.01)
- Use of cyclosporine (CsA) was associated with reduced risk of BKVN (OR 0.15, p=0.02, CI 0.032-0.74)

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Risk factors (4)

- In order to evaluate the association between level of immunosuppression and risk of BKVN, our patients were further categorized into **subgroups for analysis**
  - **Higher level of immunosuppression: received induction therapy or tacrolimus.**
  - **Lower level of immunosuppression: not receiving either of the two**
- **Significantly more patients with BKVN were under higher level of immunosuppression** (93.3% vs 56.7%, p=0.008)
- This higher level of immunosuppression was found to be associated with increased risk of BKVN (OR 10.7, p=0.026, CI 1.3-86.7)

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Number of previous acute rejection[†]	0 [0, 1]	0 [0, 0]	0.057 [b]	0 [0, 1]
History of previous acute rejection	7 (46.7)	14 (23.3)	0.106	21 (28.0)
Delayed graft function	2 (13.3)	5 (8.3)	0.622	7 (9.3)
Cytomegalovirus infection	3 (20.0)	16 (26.7)	0.747	19 (25.3)
Ureteral trauma	0 (0.0)	1 (1.7)	1.000	1 (1.3)

[a] Fisher's exact test.
[b] Mann-Whitney U test.
[c] Pearson's Chi-square test.
[†] Presented as median [quartile 1, quartile 3].
– Not applicable.

Table 2. Association between subjects' characteristics and outcome: univariate logistic regression analysis.

	OR (95% CI)[†]	*p* -values
Male gender	2.286 (0.697, 7.493)	0.172 *
Diabetes mellitus	6.000 (1.581, 22.768)	0.008 ***
Number of HLA mismatch	2.299 (1.131, 4.675)	0.021 ***
Second warm ischemic time (min)	1.063 (0.983, 1.151)	0.127 *
FK	6.948 (1.442, 33.48)	0.160 *
CsA	0.154 (0.032, 0.741)	0.020 ***
High level of immunosuppression (induction/ FK)	10.706 (1.321, 86.732)	0.026 ***
Number of previous acute rejection	3.118 (1.051, 9.251)	0.040 ***
History of previous acute rejection	2.875 (0.886, 9.334)	0.079 **

[†] Unadjusted odds ratio derived from univariate logistic regression.

* p <0.2; ** p <0.1; *** p <0.05.

Risk factors (5)

- Among the 15 patients with BKVN, 6 of them (40%) had one previous acute rejection and 1 (6.7%) had two previous acute rejections while only 14 patients (23.3%) of the control group had one previous acute rejection and none of them had more than one previous rejections
- **The number of previous acute rejection was associated with increased risk of BKVN** (OR 3.12, p=0.04, CI 1.05- 9.25).

Table 1. Demographic and clinical characteristics of subjects

	Case (n=15)	Control (n=60)	*p* -values [a]	Total (n=75)
	Count (%)	Count (%)		Count (%)
Subgroup of induction therapy			0.792	
Nil	11 (73.3)	45 (75.0)		56 (74.7)
Lymphocyte induction (ATG/ alemtuzumab)	3 (20.0)	8 (13.3)		11 (14.7)
Anti-IL2R induction	1 (6.7)	7 (11.7)		8 (10.7)
Number of immunosuppressants			1.000	
Two	0 (0.0)	2 (3.3)		2 (2.7)
Three	15 (100.0)	58 (96.7)		73 (97.3)
Immunosuppresssant used				
Prednisolone	15 (100.0)	60 (100.0)	–	75 (100.0)
Tacrolimus	13 (86.7)	29 (48.3)	0.007 [c]	42 (56.0)
Cyclosporine	2 (13.3)	30 (50.0)	0.010 [c]	32 (42.7)
Mycophenolate mofetil	13 (86.7)	51 (85.0)	1.000	64 (85.3)
Azathioprine	0 (0.0)	5 (8.3)	0.576	5 (6.7)
Sirolimus	1 (6.7)	1 (1.7)	0.362	2 (2.7)
Everolimus	0 (0.0)	2 (3.3)	1.000	2 (2.7)
Higher level of immunosuppression (induction and/or FK)	14 (93.3)	34 (56.7)	0.008 [c]	48 (64.0)
Number of previous acute rejection[†]	0 [0, 1]	0 [0, 0]	0.057 [b]	0 [0, 1]
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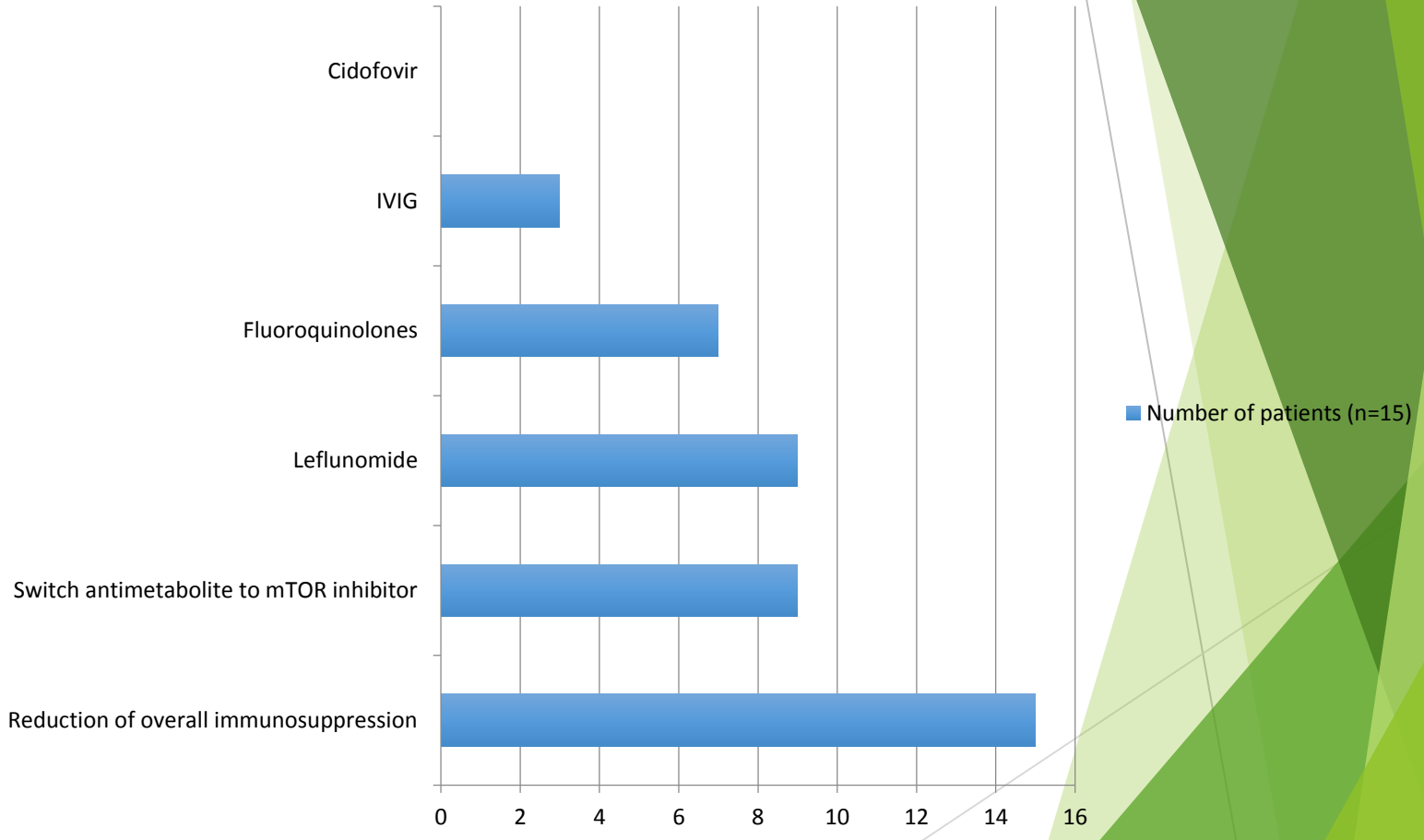
Management of BKVN

- **All of the 15 patients (100%) had reduction of overall immunosuppression as first-line treatment.**
- Adjuvant therapies were also given to 13 patients (86.7%)
- Most patients received a combination of therapies and the median number of therapies offered to patients was 3 (IQR 3-4).



Clinical course and Outcomes

- The median time from transplant to diagnosis of BKVN was 7.2 months (IQR 4.3-15.3 months)
- The median follow-up period after diagnosis of BKVN was 39.1 months (IQR 22.3 – 52.3 months)
- Three patients (patient 1, 5 and 6) (20%) had graft loss, which required resumption of dialysis
- Six patients (40%) had acute rejection after diagnosis of BKVN
- Three patients (patient 6, 9 and 12) (20%) died of sepsis during the study period.

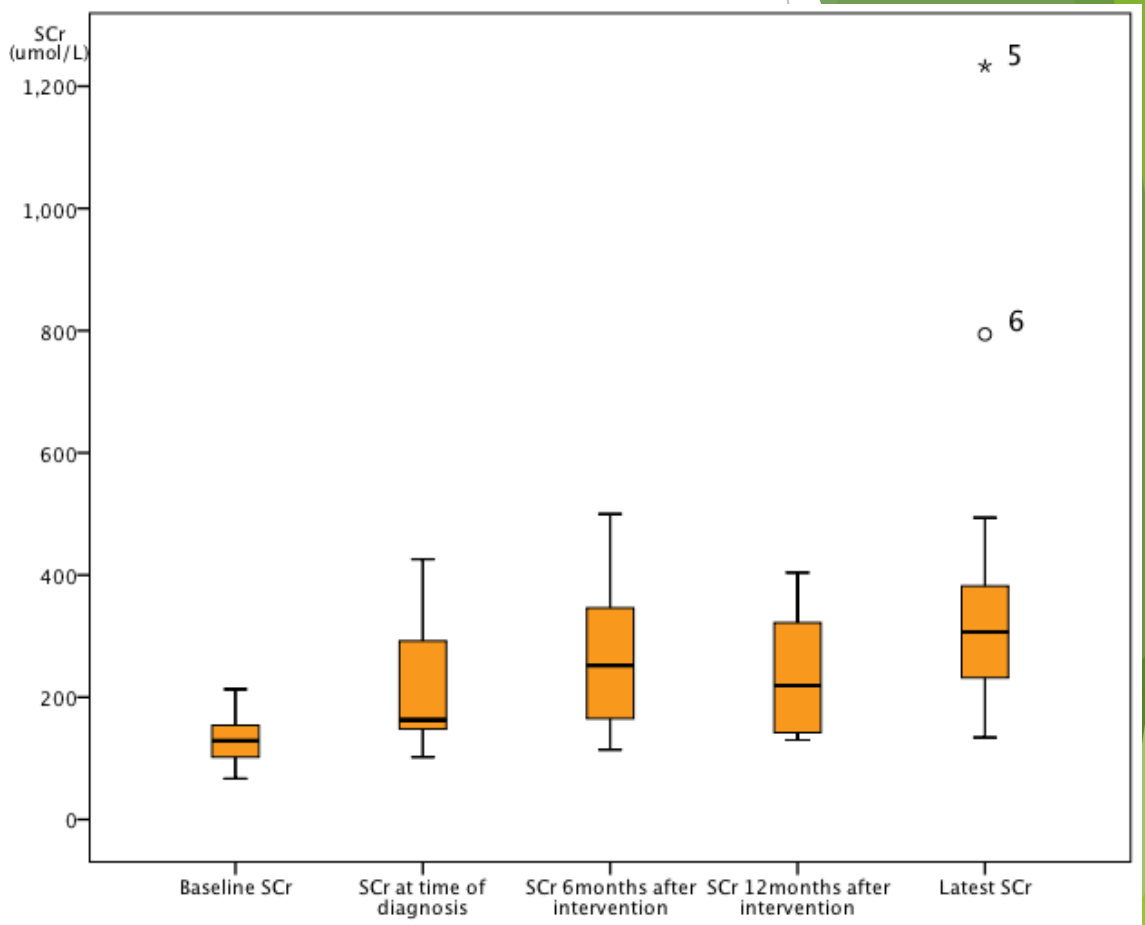
Table 4. Summary of clinical course and outcome of patients with BKVN

	Median [IQR] or Count (%)
Time from transplant to diagnosis of BKVN (months)	7.2 [4.3, 15.3]
SCr at baseline (umol/L)	123 [99, 154]
Percentage change of SCr (from baseline to latest, %)	121 [53, 246]
Graft loss	3 (20)
BKV DNA at diagnosis	9.5×10^4 [1.9×10^4, 4.5×10^5]
BKV DNA maximum level	1.1×10^5 [6816, 1.9×10^6]
Latest BKV DNA	1326 [353, 6087]
BK viremia clearance over 12months after intervention	9 (60)
Acute rejection after BKVN	6 (40)
Follow-up time after diagnosis of BKVN (months)	39.1 [22.3, 52.3]
Histology	
Banff ct score	
ct1	10 (66.7)
ct2	1 (6.7)
ct3	2 (13.3)
Drachenberg grade	
A	1 (6.7)
B	10 (66.7)
C	2 (13.3)
Polyoma viral load level	
Pvl 1	4 (26.7)
Pvl 2	1 (6.7)
Pvl 3	8 (53.3)
Death	3 (20)

SCr, serum creatinine.

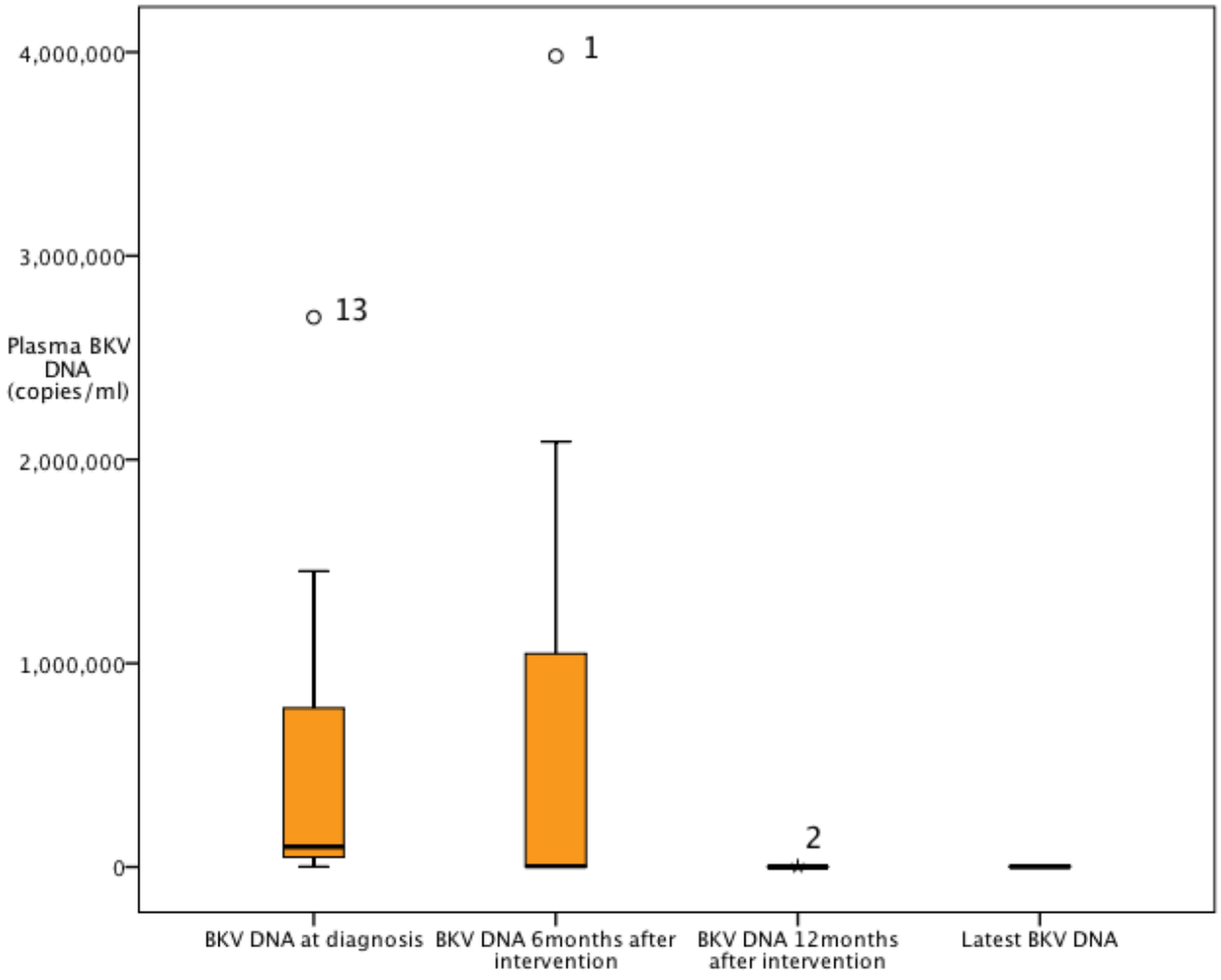
- Gradual rise of SCr was observed from baseline (median SCr 123umol/L, IQR 99-154umol/L) to time of diagnosis (median SCr 163umol/L, IQR 135-292 umol/L) and 6 months after intervention (median SCr 240 umol/L, IQR 157.3-354.5 umol/L)
- The rate of rise of creatinine then slowed down and plateaued at 12 months after intervention (median SCr 206.5 umol/L, IQR 141-333.5 umol/L).
- The median percentage change of SCr was 121% (IQR 53-246%).

Figure 2. Serum creatinine at different time intervals



5 and 6, patient 5 and 6 respectively; Both patients suffered from graft failure by the end of study.

Figure 3. Plasma BKV DNA level at different time intervals



- Plasma BKV DNA level peaked at 6months after intervention, then fell rapidly to very low level (less than 10^4 copies/ml) at 12months after intervention and remained static at that low level thereafter
- By comparing Figure 2 and 3, one could appreciate that **there was gradual decline in renal function during the course of disease despite successful suppression of plasma BKV DNA level after intervention**

Risk factors associated with worse outcome

- **Patients were grouped into those with or without doubling of SCr** by the end of study and compared for various clinical, biochemical, virological, histologic and management indices
- 7 patients did not have doubling of SCr while 8 patients had doubling of SCr
- **Patients without doubling of SCr had a significantly shorter time from transplant to diagnosis of BKVN** (median 5.0 months, IQR 3.7 – 7.2 months vs median 13.3 months, IQR 7.4 - 23.8 months, p=0.029)
- No significant differences between the two groups were observed regarding number of interventions, SCr and plasma BKV DNA at different time intervals, BK viraemia clearance, acute rejection after BKVN and different histology grading.

Table 5. Comparison of potential risk factors for outcome in patients with change in serum creatinine <100% or >100% from baseline

	change in SCr <100% (n=7) Count (%)	change in SCr >100% (n=8) Count (%)	*p* - values [a]
Time from transplant to diagnosis of BKVN (months)[†]	5.0 [3.7, 7.2]	13.3 [7.4, 23.8]	0.029 ***
Number of interventions[†]	3 [3, 4]	3 [2.25, 4.75]	0.867
SCr at baseline (umol/L)[†]	123 [102, 134]	117 [76, 164]	0.779
SCr at time of diagnosis (umol/L)†	151 [127, 216]	263 [142, 295]	0.189 *
SCr 6month after intervention (umol/L)[†]	188.5 [121, 252]	337 [187, 383]	0.590
SCr 12months after intervention (umol/L)[†]	163 [141, 209]	318 [158, 381]	0.142 *
BKV DNA at diagnosis (copies/ml)[†]	91656 [4038, 106652]	2452600 [98690, n/a]	0.670
BKV DNA maximum level (copies/ml)[†]	106652 [5901, 1081166]	2452600 [5564, 20438082]	0.432
BK viremia clearance at 6month after intervention	4 (57.1)	2 (25.0)	0.567
BK viremia clearance over 12months after intervention	5 (71.4)	3 (37.5)	0.592
BK viremia clearance at 12month after intervention	5 (71.4)	4 (50.0)	1
Acute rejection after BKVN	1 (14.3)	6 (62.5)	0.119 *
Banff ct score			0.455
ct1	5 (71.4)	4 (50.0)	
ct2	1 (14.3)	0 (0)	
ct3	0 (0)	2 (25.0)	
Drachenberg Grade			0.182
A	0 (0)	1 (12.5)	
B	6 (85.7)	3 (37.5)	
C	0 (0)	2 (25.0)	
Polyoma viral load level			1
1	2 (28.6)	2 (25.0)	
2	1 (14.3)	0 (0)	
3	3 (42.9)	3 (37.5)	

[a] Mann-Whitney U test for continuous variables; Fisher's exact test for categorical vairables.

[†]Presented as median [IQR].

* p<0.2; ** p<0.1; *** p<0.05.

Sum of percentages might not be equal to 1 because of missing data.

Limitations

Limitations

- This is a retrospective study such that some confounding variables might not be considered in the analysis
- The relatively small patient number made demonstration of significant relationship between certain risk factors and occurrence of BKVN difficult
- Some data was missing in patients who had renal transplant done in the private sector.
  - The percentage of missing data on number of HLA mismatches, cold ischemic time and second warm ischemic time were 37.3%, 58.7% and 42.7% respectively. This might lead to insufficient statistical power for the accuracy and for the analysis of the risk factors
- Although a median follow-up duration of 39.1 months is longer than some previous studies, it is still relatively short and a longer follow-up would allow better evaluation of clinical course and outcome of the disease.

Conclusion

Conclusion

- The incidence of BKVN in our centre is comparable to other studies in the literature
- Risk factors identified included history of diabetes mellitus, use of tacrolimus-combinations compared to cyclosporine-combinations, higher level of immunosuppression as defined by use of induction therapy or tacrolimus, and number of previous acute rejections
- Graft outcome is generally poor, which is consistent with other studies
- Persistent allograft dysfunction was observed despite BK viraemia clearance suggesting permanent damage to kidney after BKVN
- Early diagnosis and treatment of BKVN is crucial since a shorter time to diagnosis is the most important factor in predicting better graft outcome

Thank you!