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ORIGINAL ARTICLE

BK virus viral load: analysis of the requests received by the microbiology laboratory and clinical involvement of the issued results

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Abstract
Automation of viral diagnosis has led to an increase of BK virus (BKV) viral load (VL) requests. The aim of this study was to assess the suitability of serum creatinine (SCr) for controlling the demand and to study the clinical characteristics of BKV infection. This is a retrospective study including patients with BKV VL request during April–July 2017. Clinical records and SCr were analyzed. Five hundred samples from 333 patients were included; 61.4% of samples were from males (55.5 ± 14.8 years), and all belonged to transplant recipients (86.4% renal). BKV VL was detectable in 40 samples (8.0%) from 23 patients (6.9%), who presented high SCr (100% vs. 90.9%, $P = 0.038$). Most of detectable VLs (62.5%) belonged to patients in their first year post-transplant. Six patients with detectable VL (26.1%) developed clinical manifestations, most of them (83.3%) had a first BKV VL greater than 10,000 copies/mL ($P = 0.001$). In conclusion, SCr would be useful to identify suitable specimens for BKV VL testing without missing cases.

Keywords BK virus · Viral load · Serum creatinine · Transplantation

Introduction

A median of 19.5% of kidney transplant recipients experience BK viremia post-transplantation and these are the patients that most frequently develop BKV complications, mainly BKV-associated nephropathy (BKVAN) [1–3]. Because effective and safe antiviral therapy is lacking, screening for BKV replication (quantifying BKV DNA by PCR) has become the key recommendation to initiate and monitor an immunosuppressive treatment [3, 4]. Screening for BKV replication should be performed monthly during the first 6 months post-transplant, later, every 3 months until the first 2 years post-transplant, and then annually until the fifth year post-transplant [4, 5]. The screening should also be performed with an unexplained serum creatinine (SCr) rise or after acute rejection treatment [4, 5]. Although the indications for monitoring are clear, with the implementation of the electronic medical record and the electronic request to the laboratory we have seen how the number of BKV viral load (BKV VL) tests performed grows annually although the population subject to monitoring remains stable.

There are requests, received by the Laboratory of Virology, that can be regulated by some parameters; for example, molecular assays to detect viruses in cerebrospinal fluid can be regulated according to leukocyte and protein levels. Acceptance criteria based on these parameters allow to save time and cost [6, 7]. The objective of our study was to evaluate the adequacy of the viral load requests of BK virus that we receive in our laboratory according to the indications of the clinical guidelines, and to evaluate the role that a parameter such as the serum creatinine level can play in the modulation of the requests. In addition, we review the clinical interpretation of the issued results, the risk factors that our study population presented and the clinical manifestations that these patients developed.

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Patients and methods

A retrospective study was conducted at Hospital Universitario 12 de Octubre (Madrid, Spain), including all patients with request of BKV VL during April–July 2017. The study did not require ethics committee review because it is an analysis of suitably anonymised datasets. The BKV VL was performed in plasma by the RealStar® BKV PCR (Altona Diagnostics GmbH, Hamburg, Germany). Clinical records were reviewed to collect demographic data (age, sex, country of origin), clinical data (type of transplant, donor data, graft function, presentation of rejection, active CMV infection, clinical manifestations related to the BKV). The reason of the BKV VL request was also classified on medical check, impaired renal function, immunosuppressive therapy check, stable renal insufficiency, BKV monitoring due to a previous detectable result, combinations of the mentioned reasons, and a section of others for reasons not included in the clinical guidelines. The SCr level was analyzed in a simultaneously taken sample sent to the biochemistry laboratory. The SCr was classified according to reference values of our biochemistry laboratory (reference range, (0.50–0.90 mg/dL)). Risk factors for BKVAN were analyzed in patients with detectable VL [5, 8].

Continuous variables were compared using *t* test or the Mann-Whitney *U* test, and categorical parameters with χ^2 or Fisher's exact test. All tests were two-tailed, and *P* value < 0.05 was considered statistically significant.

Results

Five hundred samples from 333 patients were included; 61.4% of samples were from men ($n = 307$), mean age 55.5 ± 14.8 years old and all belonged to transplant recipients, mostly renal ($n = 432$; 86.4%). The other samples belonged to liver transplant ($n = 36$; 7.2%), kidney-pancreas transplant ($n = 16$; 3.2%), liver-kidney transplant ($n = 8$; 1.6%), lung transplant ($n = 2$; 0.4%), allogeneic stem cell transplant ($n = 5$, 1.0%), and kidney and autologous stem cell transplant ($n = 1$; 0.2%). Forty samples (8.0%) from 23 patients (6.9%) had detectable BKV VL. Six of them showed < 100 copies/mL BKV and the other 34 a median (interquartile range) of 1,794.50 copies/mL (435.00–8,129.50). A comparison of clinical characteristics according to the BKV VL result is shown in Table 1. On half of the samples (52.5%, $n = 21$) with a detectable VL, this result implied a change in the immunosuppressive therapy, regardless of the quantification value.

Eighteen out of 23 (78.3%) patients with detectable BKV VL were men and 2 (8.7%) had received more than one transplant. The types of transplant of the 23 patients were as follows: kidney transplant in 19 (82.6%), kidney-pancreas transplant in 2 (8.7%), liver-kidney transplant in 1 (4.3%), and allogeneic stem cell transplant in another patient. Eighteen patients (78.3%) had been transplanted in the previous year, 4 (17.4%) in the 2–5 previous years, and one before than the 5 previous years. Risk factors for BKVAN were analyzed in the 22 patients with detectable BKV VL and kidney transplant (excluding the stem cell transplant). Regarding the donor-related risk factors: 21 (95.5%) were deceased donor, mean age of the donor 42.8 ± 18.7 years old, mean cold ischemia time 16.4 ± 7.5 h, and mean HLA mismatch 4.2 ± 1.3. Regarding the recipient-related risk factors: 18 (81.8%) patients were men, mean age of 50.2 ± 18.2 years old; about the recipient origin, 18 (81.8%) were from Spain, 1 (4.5%)

Table 1 Comparison of clinical characteristics according to the BK virus viral load

Characteristic	All samples ($n = 500$)	Detectable BKV[a] viral load ($n = 40$)	Not detectable BKV viral load ($n = 460$)	*P*
Retransplant	58 (11.6%)	2 (5.0%)	56 (12.2%)	0.298
≤ 2 years post-transplant	334 (66.8%)	33 (82.5%)	301 (65.4%)	0.028*
Serum creatinine Value (mg/dL, mean ± SD)	1.88 ± 1.32	1.81 ± 0.89	1.88 ± 1.35	0.887
Increased serum creatinine	458 (91.6%)	40 (100.0%)	418 (90.9%)	0.038*
Causes of viral load request				
Medical check	193 (38.6%)	1 (2.5%)	192 (41.7%)	< 0.001*
Impaired renal function	114 (22.8%)	7 (17.5%)	107 (23.3%)	0.405
Immunosuppressive therapy check	68 (13.6%)	4 (10.0%)	64 (13.9%)	0.489
Stable renal insufficiency	37 (7.4%)	1 (2.5%)	36 (7.8%)	0.345
BKV monitoring (because of previous detectable)	32 (6.4%)	19 (47.5%)	13 (2.8%)	< 0.001
Other causes	19 (3.8%)	0 (0.0%)	19 (4.1%)	0.387
BKV monitoring and immunosuppressive therapy check	5 (1.0%)	4 (10.0%)	1 (0.2%)	< 0.001*
Impaired renal function and immunosuppressive therapy check	22 (4.4%)	0 (0.0%)	22 (4.8%)	0.243
BKV monitoring and impaired renal function	7 (1.4%)	4 (10.0%)	3 (0.7%)	0.001*
Acute rejection	3 (0.6%)	0 (0.0%)	3 (0.7%)	1.000

[a] *BKV*, BK virus

*These results are statistically significant

Table 2 Characteristics of patients with clinical manifestations of BK virus infection

	Recipient data			Transplant data				Donor data				Post-transplant period				Clinical manifestations	
Patient	Sex	Age (years)	Country of birth	Type	Number	First detectable BKV[a] viral load (copies/mL)	Time from trasplant to first detectable BKV viral loads (days)	Age (years)	Deceased	Cold ischemia time (hours)	HLA mismatch	Diabetes mellitus	Delayed graft function	CMV[b] infection	Acute rejection	Clinical manifestation	Pathological anatomy
1	Man	18	Spain	Kidney	1	11738	40	NA[c]	Yes	NA	5	No	No	Yes	No	Nephropathy	BKV nephropathy
2	Man	41	Spain	Kidney-pancreas	1	16474	17	26	Yes	16.0	5	Yes	Yes	No	No	Nephropathy	NA
3	Man	45	Spain	Kidney	2	218790	1726	18	Yes	18.0	2	No	No	No	Yes	Nephropathy	NA
4	Man	36	Spain	Kidney	1	632875	858	20	Yes	18.5	2	No	No	No	Yes	Nephropathy	NA
5	Man	68	Spain	Kidney	1	< LOQ[d]	97	59	Yes	14.2	6	No	No	No	No	Nephropathy	BKV nephropathy
6	Female	34	Paraguay	Allogeneic stem cell	1	147433	- 21[e]	43	No	NAp[f]	NAp	NAp	NAp	Yes	NAp	Hemorrhagic cystitis	NA

[a] *BKV*, BK virus

[b] *CMV*, cytomegalovirus

[c] *NA*, not available

[d] *LOQ*, limit of quantification (100 copies/mL)

[e] This patient suffered from chronic lymphocytic leukemia and was being treated with chemotherapy drugs when she had the first detectable BKV viral load, 21 days before the transplantation

[f] *NAp*, not applicable. These risk factors are not applicable for clinical manifestations different from BKV nephropathy

from Colombia, 1 from Ukraine (4.5%), 1 from Morocco (4.5%), and 1 from China. Finally, about the risk factors in the post-transplant period: 8 patients (36.4%) had diabetes mellitus, 8 delayed graft function, 6 (27.3%) cytomegalovirus infection and 5 (22.7%) acute rejection. Only in 5/22 patients (22.7%) a pathological anatomy study was carried out: BKVAN was found in 2 patients (9.1%), nonspecific renal lesions in 2 and no lesions in the other patient (4.5%). We found a correlation between the BKV VL and the presence of clinical manifestations ($P = 0.001$): 6 out of the 23 (26.1%) patients with detectable BKV VL developed clinical manifestations (5 (83.3%) had a first detectable sample with > 10,000 copies/mL). The characteristics of the 6 patients with clinical manifestations are shown in Table 2.

Discussion

All samples with detectable BKV VL were obtained from patients showing increased serum creatinine levels, as well as most samples with undetectable viral load; however, all samples belonging to patients with a normal serum creatinine were negative for BKV VL. It seems reasonable therefore, the use of serum creatinine as a parameter of the request's suitability, allowing the clinical laboratory to discriminate the requests that do not follow the established recommendations.

Most detectable samples belonged to patients in the first year post-transplant, decreasing along with years after transplantation, as it has been previously reported [5, 9]. The main reasons for the BKV VL request in detectable samples were impaired renal function and immunosuppressive therapy check.

In our study, only 26.1% of patients with detectable BKV VL developed clinical manifestations, which is a similar rate to that reported in other studies [10]. The clinical manifestations that our patients have presented (5 BKVAN in kidney transplant and 1 hemorrhagic cystitis in an allogeneic hematopoietic stem cell transplant) coincide with those found by other authors [3, 8]. Based on the study of Hirsch et al. [11] a value of 1×10^4 copies/mL of BKV has been proposed as a threshold for improving the positive predictive value of BKV VL on blood. However, there is a study that suggests that this cutoff underestimates the diagnosis of BKVAN [12] and it is a fact that standardization of the BKV VL assays is required. In addition, variant BKV strains may have a substantial effect on quantification of the VL [13]. In our study, all patients with clinical manifestations had a first BKV VL > 10,000 copies/mL, except for one patient with a first detectable BKV VL below the limit of quantification (100 copies/mL). However, reporting a detectable VL involved a change in the immunosuppressive therapy in half of the cases, regardless of the VL value obtained. A definitive diagnosis of BKVAN requires a kidney biopsy, but it is lacking in most cases [2, 9]. All patients with clinical manifestations, except for the stem cell transplant recipient, were white-ethnicity men with a deceased donor, which are important risk factors for BKVAN [8, 14].

In conclusion, although previous studies have associated high SCr level to a worse prognosis of BKV infection [10, 15], to our knowledge, this is the first time that SCr value has been analyzed as a parameter to study the suitability of BKV VL requests. Other parameters such as the time after transplantation and the reason for the request may also be useful to identify BKV VL requests that do not follow established clinical guidelines and to control unnecessary laboratory orders. Most patients (> 80%) with clinical manifestations had a first BKV VL > 10,000 copies/mL.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Vigil D, Konstantinov NK, Barry M, Harford AM, Servilla KS, Kim YH, Sun Y, Ganta K, Tzamaloukas AH (2016) BK nephropathy in the native kidneys of patients with organ transplants: clinical spectrum of BK infection. World J Transplant 6:472–504
2. Ambalathingal GR, Francis RS, Smyth MJ, Smith C, Khanna R (2017) BK polyomavirus: clinical aspects, immune regulation, and emerging therapies. Clin Microbiol Rev 30:503–528
3. Hirsch HH, Randhawa P, AST Infectious Diseases Community of Practice (2013) BK polyomavirus in solid organ transplantation. Am J Transplant 4:179–188
4. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group (2009) KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant 9(Suppl 3):S1–S155
5. Hirsch HH, Babel N, Comoli P, Friman V, Ginevri F, Jardine A, Lautenschlager I, Legendre C, Midtvedt K, Muñoz P, Randhawa P, Rinaldo CH, Wieszek A, ESCMID Study Group of Infection in Compromised Hosts (2014) European perspective on human polyomavirus infection, replication and disease in solid organ transplantation. Clin Microbiol Infect 20:74–88
6. Simko JP, Caliendo AM, Hogle K, Versalovic J (2002) Differences in laboratory findings for cerebrospinal fluid specimens obtained from patients with meningitis or encephalitis due to herpes simplex virus (HSV) documented by detection of HSV DNA. Clin Infect Dis 35:414–419
7. Hanson KE, Alexander BD, Woods C, Petti C, Reller LB (2007) Validation of laboratory screening criteria for herpes simplex virus testing of cerebrospinal fluid. J Clin Microbiol 45:721–724
8. Balba GP, Javaid B, Timpone JG (2013) BK polyomavirus infection in the renal transplant recipient. Infect Dis Clin N Am 27:271–283
9. Bechert CJ, Schnadig VJ, Payne DA, Dong J (2010) Monitoring of BK viral load in renal allograft recipients by real-time PCR assays. Am J Clin Pathol 133:242–250
10. Mohamed M, Parajuli S, Muth B, Astor BC, Panzer SE, Mandelbrot D, Zhong W, Djamali A (2016) In kidney transplant

recipients with BK polyomavirus infection, early BK nephropathy, microvascular inflammation, and serum creatinine are risk factors for graft loss. Transpl Infect Dis 18:361–371
11. Hirsch HH, Knowles W, Dickenmann M, Passweg J, Klimkait T, Mihatsch MJ, Steiger J (2002) Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. N Engl J Med 347:488–496
12. Hassan S, Mittal C, Amer S, Khalid F, Patel A, Delbusto R, Samuel L, Alangaden G, Ramesh M (2014) Currently recommended BK virus (BKV) plasma viral load cutoff of ≥4 log10/mL underestimates the diagnosis of BKV-associated nephropathy: a single transplant center experience. Transpl Infect Dis 16:55–60
13. Randhawa P, Kant J, Shapiro R, Tan H, Basu A, Luo C (2011) Impact of genomic sequence variability on quantitative PCR assays for diagnosis of polyomavirus BK infection. J Clin Microbiol 49: 4072–4076
14. Sawinski D, Goral S (2015) BK virus infection: an update on diagnosis and treatment. Nephrol Dial Transplant 30:209–217
15. Huang G, Wu LW, Yang SC, Fei JG, Deng SX, Li J, Chen GD, Fu Q, Deng RH, Qiu J, Wang CX, Chen LZ (2015) Factors influencing graft outcomes following diagnosis of polyomavirus - associated nephropathy after renal transplantation. PLoS One 10:e0142460