Prospective study of BKV nephropathy in 117 renal transplant recipients

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──── SUMMARY ────

Nephropathy caused by poliomavirus (BKVAN) in transplant recipients is responsible for the loss of the transplanted organ. In this study we suggest a non-invasive diagnostic protocol for the early identification of BKVAN during follow-up treatments. In 117 kidney transplant recipients follow-up was performed every three months during a two year period after transplantation and a positive screening result was confirmed and assessed by quantitative assays (BKV DNA load in plasma and urine). The definitive diagnosis of BKV requires allograft biopsy. Of the 117 patients 4 had BKVAN (3.4%), and the consequential reduction of immunosuppression improved kidney function and plasma clearance of the virus was achieved.

KEY WORDS: BKV, nephropathy, diagnosis

INTRODUCTION

BKV is a double-stranded DNA virus, which belongs to the Papova family together with JCV and SV 40. BKV and JCV both infect humans. In fact, a high percentage (80% ranging from 46 to 94%) of adults are seropositive towards these viruses and the percentages of viral reactivations expressed as positive DNAemias are 0-20% in the normal population and 50-60% in immunocompromised patients, respectively. In transplant recipients, BKV can cause a tubular interstitial nephropathy (BKVAN) with an incidence of approximately 5% and it can be the cause of premature loss of the transplanted organ (De Bruyn, *et al.,* 2004). It seems almost certain that the correlation between BKVAN and kidney transplant depends mostly upon the reactivation of the latent virus (kidney and/or ureter and/or bladder) following immunosuppressive therapy. When a nephropathy due to BKV is suspected a kidney biopsy is the gold standard for a precise diagnosis. Results obtained by cytological techniques (search for decoy cells in urine) and mostly biomolecular testing for viral DNA (DNA BKV) in urine (viruria) and in serum (viremia) could be very important non-invasive tools in the early diagnosis of BKVAN (Brahm, *et al.,* 2005; Pietropaolo *et al.,* 2005) during post transplant follow-up. In this study we suggest a non-invasive diagnostic protocol for BKVAN diagnosis in kidney transplant recipients.

MATERIALS AND METHODS

Patients
From January 2003 to December 2005, 117 kidney transplant recipients (77 males and 40

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females) (mean age: 52.6) underwent post-transplant follow-up for BKV nephropathy. Among these patients, 41 (35%) were treated with tacrolimus (FK) and mycophenolate mofetile (MMF)**,** 2 (1.8%) with rapamycin (Sirolimus) and MMF and 74 (63.2%) with cyclosporine (CyA) and MMF. Of the 117 patients, 5 patients (5.8%) had undergone a transplant from a live donor, the remaining 112 patients (94.2%) received a kidney from a cadaver.

BKV screening

In all of the patients, a screening for BKV DNA in blood and urine, and urinary cytology (search for decoy cells) was performed every three months during a two year period after transplantation. Urinary cytology was performed with Papanicolaou's method to evaluate the presence or absence of viral intranuclear inclusions (decoy-cells) (Drachenberg, *et. al,* 2001). Urine and blood samples were kept at +2/+8°C for a maximum of 4 hours, or frozen at –20°C for a maximum of 30 days. Samples were frozen at –70°C until used.

Viral DNA

DNA was extracted from urine and blood samples by EXTRAGEN (Nanogen Advanced Diagnostics s.r.l.) which is able to detect DNA or RNA in free viral particles. The extraction procedure was carried out according to the manufacturer's instructions, and sample lysis performed with a chaotropic reagent (guanidine hydrochloride), a detergent (CTAB) and a reducing agent (2-mercaptoethanol). After protein precipitation at high temperature, nucleic acids, after ethanol washing (70%) were dissolved in ultrapure water.

Nested and Real-Time PCR

All the samples which resulted positive for BKV DNA screening revealed through nested PCR techniques, underwent a quantitative evaluation, using a Real-Time duplex technique (Nanogen Advanced Diagnostics s.r.l.). The Real-Time kits (Nanogen Advanced Diagnostics s.r.l.) were performed upon Applied Biosystem instruments series 7000, and used a specific amplification for the large T Antigen BKV gene. Moreover the region of the human beta globulin gene was used as an internal control for the sample being tested. For the determination of the DNA titer it is necessary to perform some reactions with standard DNA having known concentrations (four point titer).

Kidney biopsy

After informed consensus, a kidney biopsy was obtained from four patients. The presence of viral inclusions, in the epithelium of renal tubules, were detectable after hematoxylin eosin staining, PAS, and trichromic staining. More immunohistochemical techniques using monoclonal antibodies were performed for the search of large T SV40 antigens (Randhawa, *et al.*, 2003). In these four patients histological, immunohistochemistry, ultrastructural or seroimmunological examinations were performed for other viral infections (cytomegalovirus, Epstein-Barr virus, Herpes virus and adenovirus).

RESULTS

The renal biopsy samples for BKV contained viral intranuclear inclusions in the epithelium cells of the kidney tubules. The patients were divided into 4 groups on the basis of BKV positivity in blood and urine. In the 24 month follow-up period (Table 1), 72 patients (61.5%) were negative for the virus as established by absence of decoy cells and the absence of viruria and viremia.

TABLE 1 - *BKV DNA in urine and plasma of 117 kidney transplant recipients by PCR and nested Real-Time PCR.*

Patients with negative viruria and viremia		Patients with positive (from 10^3 to >10^6 cp/ml) viruria and negative viremia		Patients with negative viruria and positive viremia (from 10^2 to 10^4 cp/ml)		Patients with positive (from 10^2 to >10^6 cp/ml) viruria and positive viremia (from 10^2 to >10^6 cp/ml)	
n.	*%*	*n.*	*%*	*n.*	*%*	*n.*	*%*
72	61.5	27	23.1	3	2.6	15	12.8

TABLE 2 - *BKV viral load in urine and serum of kidney transplant recipients with and without BKV nephropathy (No BKVNA and BKVNA)*

Kidney transplant recipients without BKV nephropathy		Kidney transplant recipients with BKV nephropathy	
Positive Viruria (from 10^2 to $>10^6$ cp/ml) and viremia (10^2-10^4 cp/ml)		Positive Viruria (from 10^2 to $>10^6$ cp/ml) and viremia ($>10^4$ cp/ml)	
n.	%	n.	%
11	9.4	4	3.4

On the other hand, in 27 patients (23.1%) a persistent and constant viruria was present during the 2 year follow-up (range: 10^3->10^6 cp/ml) and in 3 patients (2.6%) only a temporary viremia (range 10^2-10^4 cp/ml). 15 patients (12.8%) had persistent viruria and viremia in the follow-up. Of these 15 patients, eleven (9.4%) presented different ranges of viruria (10^2->10^6 cp/ml) always correlated with viremia $<10^4$ cp/ml; none of the 11 patients had an increase in serum creatinine concentration or a deterioration of kidney function during the 24 month follow-up. Four males out of 15 (3.4%) had a viremia which resulted greater than 10.000 cp/ml and virurias greater than 1.000.000 cp/ml (Table 2).

In this last group of patients, a positive urinary cytology for decoy cells was present from the sixth month of observation. During follow-up the values of creatinine increased and kidney functionality deteriorated. Therefore these 4 patients underwent a kidney biopsy. The histological examination and the immunohistochemistry technique for the presence of the SV 40 antigen confirmed the clinical suspicion of BKVAN. The kidney biopsies of the four patients revealed intranuclear viral inclusions in the epithelium cells of the kidney tubules. Histological, immunohistochemical, ultrastructural and seroimmunological examinations failed to reveal the presence of other viral infections (Cytomegalovirus, Epstein-Barr virus, Herpes virus and Adenovirus) in these 4 patients. At the time of BKVAN diagnosis the four patients were treated with the following immunosuppresive therapy protocols: MMF-CyA-prednisone (one patient) and FK-MMF-prednisone (three patients). A deterioration of renal function was noted in all patients 14.6±6.1 months after transplant. The suspension of cortisone which was administered initially to avoid a rejection, together with modifications of the immunosuppressive therapy led to a better kidney functionality, a gradual and progressive disappearance of the virus in circulation and decrease in urinary viral load and in the number of decoy cells.

DISCUSSION

BKVAN is associated to a premature loss of the functionality of the organ in patients who had a kidney transplant (Bergallo *et al.,* 2002; Hariharan, 2006). We do not have precise evaluations on the incidence of BKVAN even if various authors report that the virus is present in 2 to 10% of patients who undergo a kidney transplant (Hirsch HH, *et al.,* 2002). The clinical experience acquired and the outcome of disease after changing immunosuppressive therapy suggests that tubular interstitial nephropathy might be due to over-immunosuppression, but significant epidemiological factors have not been found to date (Binet, *et al.,* 1999). This makes the clinician's work more difficult because the reduction of immunosuppressive therapy can improve the natural history of BKVAN, but it could also determine an acute rejection (Celik, *et al.,* 2003).

Even if kidney biopsy has been considered the gold standard for the diagnosis of BKV nephropathy, the use of non-invasive techniques such as the search for decoy cells, and the qualitative and quantitative determinations of BKV DNA in serum and urine might help us in selecting patients at greater risk of suffering from a nephropathy which would lead to loss of the organ (Vera-Sempere, *et al.,* 2005). But these methods cannot substitute the biopsy. The presence of decoy cells in urine is a sign of viral replication, but its use is limited due to the low sensitivity and specificity and rapid deterioration of cells in urine

(a few minutes). Therefore this test cannot be used in routine screening. Literature data demonstrated that 100% of transplant recipients with tubular interstitial nephropathy and positive viremia always have a positive urinary PCR result. This proves that the search for viral DNA in urine has a very high sensitivity even if it is not specific for BKVAN and has a positive predictive value of only 30% (Nickeleit, *et al.*, 1999). The BK viral load in plasma (cut-off 10.000 copies /ml) has a sensitivity of 92% and a 100% specificity. It also has a 60% positive predictive value and a 95% negative predictive value (Hirsch and Steiger 2003). Our protocol for the rapid and early identification of BKVAN in patients, who underwent a kidney transplant needs periodic monitoring for the presence of decoy cells and the determination of urinary and plasma BKV DNA. This allowed the selection of 15 patients (12.8%) with viruria and viremia. Four of these patients had a plasma viral load greater than 10.000 cp/ml and urinary viral load greater than 1.000.000 cp/ml. The possibility to monitor the viral reactivation of BKV helped these patients (3.4%) in having an early diagnosis of their nephropathy by undergoing a kidney biopsy. This last group of patients had the presence of decoy cells, high urinary and plasmatic viral loads and an alteration of kidney function. Early diagnosis suggested a reduction of immunosuppressive therapy which then caused a stabilization of nephropathy and an improvement of kidney function. This result encourages us to suggest persevering with this strategy of screening to obtain an early diagnosis of patients at risk. With early diagnosis immunosuppressive therapy is modified, and BKVAN patients monitored. All of these factors contribute to achieve a better outcome from a kidney transplant (Kiberd, 2005). Screening is not cheap, but in cases of BKV nephropathies it is justified by the disease that can be contrasted only by an early diagnosis of viruria and viremia which must be monitored to avoid organ loss.

REFERENCES

BERGALLO, M., MERLINO, C., BOLLERO, C., SCUTERA, S., SINESI F., CAVALLO R. (2002). Human polyoma virus BK DNA detection by nested PCR in renal transplant recipients. *New Microbiol.* **25** (3): 331-4.

BINET, I., NICKELEIT, V., HIRSCH, HH., PRINCE, O., DALQUEN, P., GUDAT, F., MIHATSCH, M,J., THIEL, G. (1999). Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. *Transplantation.* **27**, 67 (6): 918-22.

KIBERD, BRYCE A. (2005). Screening to prevent Polyoma Virus Nephropathy: a medical decision analysis. *American J of Transplant.* **5**, 2410-6.

BRAHM, V., ET AL. (2005). BK virus nephritis: Risk factors, timing, and outcome in renal transplant recipients. *Kidney Int.* Vol **68**, 1834-9.

CELIK, B., SHAPIRO, R., VATS, A., RANDHAWA, PS. (2003). Polyomavirus allograft nephropathy: sequential assessment of histologic viral load, tubulitis, and graft function following changes in immunosuppression. *Am. J. Transplant.* **3** (11): 1378-82.

DE BRUYN, G. AND LIMAYE, A.P. (2004). BK virus associated nephropathy in kidney transplant recipients. *Rev. Med. Virol.* **14**, 193-205.

DRACHENBERG, RC., ET. AL. (2001). Morphological spectrum of polyoma virus disease in renal allografts: diagnostic accuracy of urine cytology. *Am. J. Transplant.* **1** (4): 373-81.

HARIHARAN, S. (2006). BK virus nephritis after renal transplantation. *Kidney International.* **69**, 655-662.

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.* **15**, 347 (7): 488-96.

HIRSCH, HH. AND STEIGER, J. (2003). Polyomavirus BK. *The Lancet Infection Disease.* Vol. 3.

NICKELEIT, V., HIRSCH, HH., BINET, IF., GUDAT, F., PRINCE, O., DALQUEN, P., THIEL, G., MIHATSCH, MJ. (1999). Polyomavirus infection of renal allograft recipients: from latent infection to manifest disease. *J. Am. Soc. Nephrol.* **10** (5): 1080-9.

PIETROPAOLO, V., FIORITI, D., MISCHITELLI, M., ANZIVINO, E., SANTINI, M., MILLEFIORINI, E., DI REZZE, S., DEGENER, AM. (2005). Detection of human herpesviruses and polyomaviruses DNA in a group of patients with relapsing-remitting multiple sclerosis. *New Microbiol.* **28** (3): 199-203.

RANDHAWA, P. ZYGMUNT, D., SHAPIRO, R., VATS, A., WECK, K., SWALSKY, P., FINKELSTEIN S. (2003). Viral regulatory region sequence variations in kidney tissue obtained from patients with BK virus nephropathy. *Kidney Int.* **64** (2): 743-7.

VERA-SEMPERE, FJ., RUBIO, L., MORENO-BAYLACH, MJ., GARCIA, A., PRIETO, M., CAMANAS, A., MAYORDOMO, F., SANCHEZ-PLUMED, J., BENEYTO, I., RAMOS, D., ZAMORA, I., SIMON, J. (2005). Polymerase chain reaction detection of BK virus and monitoring of BK nephropathy in renal transplant recipients at the University Hospital LA Fe. *Transplant. Proc.* **37** (9): 3770-3.