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Clearance of BK Virus Nephropathy by Combination Antiviral Therapy With Intravenous Immunoglobulin

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Background. Reactivation of BK polyoma virus causes a destructive virus allograft nephropathy (BKVAN) with graft loss in 46%. Treatment options are limited to reduced immunosuppression and largely ineffective antiviral agents. Some studies suggest benefit from intravenous immunoglobulin (IVIG). **Methods.** We evaluated effectiveness of adjuvant IVIG to eliminate virus from blood and tissue, in a retrospective, single-center cohort study, against standard-of-care controls. Both groups underwent reduced immunosuppression; conversion of tacrolimus to cyclosporine; and mycophenolate to leflunomide, oral ciprofloxacin, and intravenous cidofovir. **Results.** Biopsy-proven BKVAN occurred in 50 kidneys at 7 (median interquartile range, 3-12) months after transplantation, predominantly as histological stage B (92%), diagnosed following by dysfunction in 46%, screening viremia in 20%, and protocol biopsy in 34%. After treatment, mean viral loads fell from 1581 ± 4220 × 10^3 copies at diagnosis to 1434 ± 70 639 midtreatment, and 0.138 ± 0.331 after 3 months ($P < 0.001$). IVIG at 1.01 ± 0.18 g/kg was given to 22 (44%) patients. The IVIG group more effectively cleared viremia (hazard ratio, 3.68; 95% confidence interval, 1.56-8.68; $P = 0.003$) and BK immunohistochemistry from repeated tissue sampling (hazard ratio, 2.24; 95% confidence interval, 1.09-4.58; $P = 0.028$), and resulted in faster (11.3 ± 10.4 months vs 29.1 ± 31.8 months, $P = 0.015$) and more complete resolution of viremia (33.3% vs 77.3%, $P = 0.044$). Numerically, fewer graft losses occurred with IVIG (27.3% vs 53.6% for control, $P = 0.06$), although graft and patient survivals were not statistically different. Acute renal dysfunction requiring pulse corticosteroid was common (59.1% vs 78.6%, $P = 0.09$), respectively, after immunosuppression reduction. **Conclusions.** Combination treatment incorporating adjuvant IVIG was more effective eliminating virus from BKVAN, compared with conventional therapy. Validation by multicenter randomized trial is needed.

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BK virus is a human polyoma virus of high prevalence and low morbidity in normal individuals, which persists in renal cortex and medulla of the transplanted kidney. Reactivation of infection in transplanted kidneys is the unintended consequence of the powerful antirejection agents, and its incidence has increased with modern immunosuppression.[1,2] Early detection of virus by programmed screening[3,4] allows reduction of immunosuppression and reconstitution of recipient immunity and potential viral clearance. However, established BK virus allograft nephropathy (BKVAN) is a destructive allograft infection that occurs in 5% and progresses to transplant failure in 46%.[1,5]

Inflammatory BKVAN (pattern B histopathology) is characterized by viral cytopathic tubular destruction, interstitial fibrosis and interstitial cellular infiltration, with graft loss rates of 25% to 75%, depending on extent of inflammation (subclassified as B1, B2, and B3).[6,7] Treatment is problematical,[3,4] because reduction of immunosuppression recommended by consensus guidelines is prejudiced by viral-mediated interstitial inflammation, often morphologically indistinguishable from acute rejection.[8-10] Available antiviral treatments for severe BKVAN are relatively ineffectual. A large systematic review of 40 studies found that the death-censored graft loss rate for immunosuppression reduction was not greatly improved by the addition of cidofovir or leflunomide.[1] Guidelines are hampered by a poor evidence base and lack of

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randomized control trials,[10,11] and better evidence-based treatment options are desperately needed.

Intravenous immunoglobulin (IVIG) is a nondepleting agent, with antiviral and immunomodulatory properties, advocated for treatment of BKVAN. IVIG is familiar to transplant clinicians for acute rejection,[12] but also successfully used against chronic viral infections such as post-transplant cytomegalovirus, Epstein-Barr virus, and parvovirus B19 infections.[13-15] Promising recent reports of IVIG used as salvage therapy for BKVAN[16-20] are small and lack control groups. Concomitant reduced immunosuppression confounds clinical interpretation, more data are needed. The purpose of this single-center retrospective cohort study was to evaluate the efficacy of IVIG, when added to a multidimensional antiviral strategy to treat established BKVAN in kidney transplant recipients. We used a combination of treatments including ciprofloxacin, conversions of tacrolimus to cyclosporine, and mycophenolate to leflunomide (with therapeutic monitoring and dose adjustment), intravenous cidofovir—to which additional IVIG was used over a 3-month treatment period. This combinatorial approach with adjuvant IVIG appeared significantly more effective clearing BK virus from blood and renal transplant tissue as the primary endpoints.

MATERIALS AND METHODS

Study Population

The study group comprised consecutive kidney and kidney-pancreas transplant recipients diagnosed with histologically-proven BKVAN from 2002 to 2014, where accessible data were available. All patients were treated at Westmead Hospital, Sydney. The institutional ethics approvals were HREC 2012/6/6.4 (3563) and LNR/12/WMEAD/244 (amendment 1). Immunosuppression for standard-risk patients comprised basiliximab induction, tacrolimus, mycophenolate, and prednisolone.

Diagnosis of BKVAN

BKVAN was confirmed by abnormal renal transplant pathology in all cases, characterized by intranuclear inclusions, typical smudgy nuclear chromatin, cellular atypia, tubular epithelial cell degeneration with rounding, detachment, and apoptosis, and supported in all cases by positive simian virus 40 T (SV40T) immunohistochemistry (and in some early case by electron microscopy). The categories of BKVAN pathology were classified by extent of disease and severity of associated inflammation and damage.[6,7]

BK viremia was present in all cases. DNA was confirmed as BK virus DNA after nucleic acid extraction from whole blood and specific quantitative real-time PCR (Affigene BKV trender kit; Cepheid AB, Solna, Sweden), with a quantitative range 10^3 to 10^8 copies/mL, (assay limit, 898 copies/mL). From July 2013, BK detection used the specific *Iam* BKV kit with LIASION analyser (DiaSorin, Vercelli, Italy), with a sensitivity of 450 copies/mL (quantitative range, 10^4-10^{10} copies/mL). Quantitative tests successfully complied with independent external quality assurance in molecular diagnostics (www.qcmd.org). Prior limited sampling (n = 19) demonstrated 16% type 1A/1C, 63% 1B, 16% II, and 5% type IV BK genotypes in our viremia-transplanted population.

From 2007 to 2008, we initiated consensus screening guidelines at 1, 2, 3, 6, 9, and 12 months posttransplantation for viremia.[10] Protocol histopathology was obtained at implantation, 1, 3, and 12 months posttransplant. After BKVAN diagnosis, repeat pathology at 3 months was frequently supplemented by indication biopsy samples and SV40 staining. Tissue viral load was assessed by a 3-tier score (1, ≤1%; 2, >1%-10%; 3, >10% tubules with viral replication), for both SV40 staining and cytopathic changes.[21] Presumptive cases of high-level BK viremia (>10^4 copies/mL) without biopsy confirmation were not included.

Treatment of BKVAN

Treatment of established BKVAN entailed reduced immunosuppression, with tacrolimus reduction or conversion to cyclosporine (2-hour target concentration, 600-800 ng/mL), reduced mycophenolate or replacement by leflunomide (60 mg/d load for 2 days then 20 mg/d; targeting plasma teriflunomide, 50-100 μg/mL)[22] or azathioprine, and prednisolone tapering to 10 mg per day. Additional therapies included ciprofloxacin (500 mg twice daily for 1 month), IV cidofovir (0.5 mg/kg per second weekly for 10 weeks), and IVIG, initially reserved for severe inflammatory BKVAN infection, but later expanded to include primary treatment of less severe disease.

IVIG was given weekly at 100 mg/kg per dose for 10 weeks (totalling 1 g/kg), using 1 L normal saline prehydration and predose enoxaparin 1 mg/kg subcutaneously to prevent thrombosis, unless contraindicated. The IVIG products used included Octagam 5% and 10% (Octapharma, Vienna, Austria), Flebogamma 5% (Instituto Grifol, Barcelona, Spain), Kiovig 10% (Baxter AG, Vienna, Belgium), Intragam P6% and Privigen 10% (both CSL Behring, Broadmeadows, Australia).

Statistical Analysis

The research design was a retrospective, case-control historical cohort study. The principal treatment group were patients who received IVIG as part of BKVAN treatment. The control group received standard-of-care treatment without IVIG.

The prespecified primary outcomes were clearance of viremia, and tissue clearance defined by resolution of cytopathic effect and SV40 on repeat histopathology. Secondary outcomes were death-censored graft survival, patient survival, and adverse events. Sustained viral response (SVR) was defined as complete clearance of BK viremia by repeated blood testing, partial clearance as low-level persistent viremia (1E + 03 copies/mL or less, sometimes intermittently), and failed clearance with high-level viremia (when quantification exceeded 1E + 04 copies/mL).

STROBE reporting guidelines cohort studies were used.[23] We used an unpaired Student *t* test or Wilcoxon rank sum test for nominal data, conditional binomial test for categorical data, Pearson and Spearman coefficients for correlations, logistic regression for dichotomous data analysis, and Cox regression for survival data (both univariate and multivariate models). Multivariate models were constructed after backward elimination to adjust for confounding factors at patient entry or during treatment of BKVAN. Data are expressed as mean ± SD unless otherwise stated. All *P* values were 2-sided, and a probability below 0.05 was considered significant.

RESULTS

Study Cohort and Baseline Information

The 50 BKVAN cases from 48 patients, included 2 men whose first transplant failed from late BKVAN and

TABLE 1.

Clinical and histology features at BKVAN diagnosis, stratified by use of IVIG therapy

Factor	Conventional	IVIG	*P*
Number	28	22	
Months posttransplant of diagnosis	19.4 ± 28.8	9.0 ± 9.2	
Median (IQR)	8 (3-19.5)	6 (3-10)	NS (0.19)
Serum creatinine, μmol/L	184 ± 54	164 ± 52	NS (0.25)
Method of detection of BKVAN			
BFC total, n (%)	16 (60.7%)	17 (77.3%)	NS (0.14)
BFC dysfunction, n (%)	13 (46.4%)	10 (45.5%)	NS (0.94)
BFC viremia, n (%)	3 (10.7%)	7 (31.8%)	NS (0.07)
Protocol biopsy, n (%)	12 (42.9%)	5 (22.7%)	NS (0.14)
Screen total, n (%)	15 (53.6%)	12 (54.5%)	NS (0.94)
Renal pathology and viral load at BKVAN diagnosis			
Banff i score	1.4 ± 0.9	1.5 ± 1.2	15 (53.6%)
Banff ci score	1.1 ± 0.9	0.8 ± 0.6	NS (0.14)
Banff ct score	1.3 ± 0.8	1.0 ± 0.6	NS (0.23)
SV40T extent	1.5 ± 0.7	1.3 ± 0.6	NS (0.32)
Quantitative BK level ($\times 10^6$ copies/mL)	2.85 ± 6.15	0.72 ± 1.83	NS (0.83)
Ln BK NAT (ln copies/mL)	10.97 ± 4.64	11.27 ± 2.93	NS (0.81)
Urinary decoy cell (%)	85.7%	95%	NS (0.43)

Mean ± SD.

BFC, biopsy for cause or indication biopsy; NAT, nucleic acid test.

whose second graft became reinfected (despite graft nephrectomy in one). Patients received 42 kidney and 8 kidney-pancreas transplants and were followed up for a median 60 months (interquartile range [IQR], 31-112) after transplantation.

Recipients age was 46.9 ± 11.8 years, 80% were men, HLA mismatch was 3.8 ± 1.7 (of 6), 32% were from living donors, and 18% were regrafts (Table 1, **Table S1, SDC,** http://links.lww.com/TXD/A34). Induction therapy was basiliximab in 52%, thymoglobulin or desensitisation in 14%, and none in 34%. Before BKVAN diagnosis, 8% of recipients required dialysis for delayed graft function, 22% experienced acute T cell rejection, and antibody-mediated rejection occurred in 4%. Antithymocyte globulin was given in 16.3% (for rejection or induction), and pulse methylprednisolone was used in 38.8% for acute (or subclinical) rejection. Pretransplant DSA were present in 14%.

Triple-therapy immunosuppression comprised tacrolimus in 94% (2 clinical trial patients substituted sirolimus or tofacinib); mycophenolate (90%) or azathioprine (6%); and prednisolone, initiated at 20 mg/d then tapered to 10 mg by 3 to 6 months. The mean daily dose of tacrolimus was 4.0 ± 2.9 mg/d (trough level, 6.4 ± 3.0 ng/mL), mycophenolate of 1.77 ± 0.45 g, and prednisolone of 14.2 ± 5.7 mg before BKVAN. There were no significant differences in demographic features, age, recipient sex, HLA mismatch, prior antibody status, transplant immunosuppression, incidences of delayed graft function or acute rejection, the severity of transplant histopathology or viral load at BKVAN diagnosis, renal function, or therapeutic levels between treatment groups of therapy with IVIG and conventional standard of care (Table 1 and **Table S1, SDC,** http://links.lww.com/TXD/A34 for additional details). More retransplanted patients occurred in the conventional group by chance (28.6% vs 4.5% for IVIG, $P = 0.03$).

The Pathological Diagnosis of BKVAN

All cases of BKVAN were verified by pathology and occurred 7 months after transplantation (median IQR, 3-12; range, 2-121), and 76% within the first year. The number of diagnostic "indication" biopsies (including for viremia) were 33 (66%), with 17 (34%) in unsuspected cases discovered by protocol screening for subclinical rejection. Biopsy indications were graft dysfunction (serum creatinine, 189 ± 49 μmol/L) in 23 (46%) and high-level viremia in 10 (20%). Screening biopsy and blood testing found 27 (54%) of BKVAN cases.

The initial pathological categories of BKVAN (n = 50) were stages A (early changes without tubular cell necrosis) in 3 (6%); B1 (active nephropathy with virally tubular necrosis) in 19 (38%), B2 in 8 (16%) and B3 in 19 (38%, B3), and late sclerosing stage C in 1 (2%). The pathological diagnosis was supported by viremia in all with a mean viral load of $1581 \pm 4220 \times 10^3$ copies/ml ($\log_e$, 11.45 ± 3.64). Shedding of "decoy cells" occurred in 92.6% of tested urine samples.

Treatments Given for BKVAN

After diagnosis, calcineurin inhibitor doses were altered in all except 5 (10%), with active inflammation. The tacrolimus dose was reduced in 11 (22%, more in conventional arm, $P = 0.007$), or replaced by cyclosporine in 26 (52%, more commonly with IVIG group, $P < 0.001$) or sirolimus in 8 (16%) of patients. Mycophenolate was converted to leflunomide in 41 (82%) at 21.7 ± 5.4 mg/d, achieving plasma teriflunomide levels of 55.8 ± 38.5 μg/mL. Leukopenia occurred with leflunomide (n = 2) and azathioprine (n = 1). Prednisolone doses of 14.2 ± 5.7 mg/d were reduced in 21 (42%) patients to 11.6 ± 4.9 mg/d (detailed in Table 2). Salvage IVIG was used in 7 (31.8%) patients after conventional therapy failed.

Ciprofloxacin (or initially gatifloxacin) was given to 45 (90%) of the patients. Intravenous cidofovir was used in 84% of BKVAN patients (at 43.4 ± 19.9 mg/dose,

TABLE 2.

Alterations to immunosuppression and ancillary treatment of BKVAN, stratified by use of IVIG with the therapy regime

Factor	Conventional	IVIG	*P*
Number	28	22	
Therapy			
Tacrolimus no change, n (%)	5 (17.8)	0	0.039
Tacrolimus reduction, n (%)	10 (36.3)	1 (4.5)	0.007
Tacrolimus to cyclosporine, n (%)	7 (25.0)	19 (86.3)	<0.001
Tacrolimus to sirolimus, n (%)	6 (21.4)	2 (9)	NS (0.24)
MMF no change, n (%)	1 (3.5)	0	NS (0.37)
MMF reduction, n (%)	3 (10.7)	2 (4.5)	NS (0.85)
MMF to azathioprine, n (%)	2 (7.1)	0	NS (0.32)
MMF to leflunomide, n (%)	21 (75)	20 (90.9)	NS (0.15)
MMF stopped, n (%)	1 (3.5)	0	NS (0.37)
Prednisolone reduction, n (%)	9 (32.1)	12 (54.5)	NS (0.12)
Any sirolimus use, n (%)	10 (35.7)	2 (9)	0.03
Ciprofloxacin, n (%)	28 (100)	17 (77.3)	NS (0.54)
Cidofovir, n (%)	22 (78.65)	20 (90.9)	NS (0.24)
IVIG therapy	0	22	<0.001

Mean ± SD.

MMF, mycophenolate mofetil.

second-weekly for 5.7 ± 1.3 infusions over 2.64 ± 0.89 months), producing 1 case of cidofovir nephrotoxicity. IVIG was used in 22 patients (44% of BKVAN), at 8.26 ± 2.67 g weekly for 2.6 ± 0.58 months, totaling 1.03 ± 0.18 g/kg, and deep vein thrombosis occurred in 1 patient (2%).

Sequential Renal Transplant Histopathology Outcomes

Tubulointerstitial inflammation occurred in 19 (38%) at BKVAN diagnosis. The mean Banff i score of mononuclear inflammation was 1.42 ± 1.03 and had increased relative to prior protocol biopsy of 0.27 ± 0.57 ($P < 0.001$), frequently maintaining activity at scores of 1.20 ± 1.04 after 3 months of treatment, and 1.3 ± 1.2 at the last evaluable renal tissue, including 12 graft nephrectomies for failed allografts (11 with inflammatory symptoms and 1 to clear viral sanctuary sites prior to retransplantation). Progressive tubulointerstitial destruction with fibrosis followed the inflammation. The mean Banff tubular atrophy (ct) scores increased from preceding protocol biopsy of 0.47 ± 0.62; to 1.14 ± 0.73 at diagnosis of BKVAN; to 1.30 ± 0.91 after 3 months treatment; and finally, to 1.74 ± 0.91 at the last test. Chronic interstitial fibrosis scores followed a similar course from 0.47 ± 0.58; to 0.96 ± 0.78 at diagnosis; 1.32 ± 0.94 at 3 months, to 1.76 ± 1.03 at the last biopsy.

The semiquantitative assessment of virus infection from SV40 immunohistochemistry and histology correlated with the natural logarithm of viral load at diagnosis ($r = 0.368$, $P = 0.035$). The histological extent of BKVAN fell from 1.42 ± 0.64 at diagnosis, to 0.87 ± 0.86 after 3 months therapy, to 0.625 ± 1.0 at final biopsy, including graft failures from BKVAN. The severity of inflammation (Banff i score) at diagnosis correlated with SV40 staining (r = 0.314,

TABLE 3.

Clinical and histological outcomes for BKVAN, stratified by use of IVIG therapy

Factor	Conventional	IVIG	*P*
Number	28	22	
Renal transplant histology at 3 mo at later			
Banff i score inflammation	1.4 ± 0.9	1.0 ± 1.1	NS (0.14)
Banff ci fibrosis score	1.5 ± 0.9	1.1 ± 1.0	NS (0.11)
Banff ct atrophy score	1.4 ± 0.9	1.2 ± 0.9	NS (0.37)
SV40T histology	1.2 ± 0.9	0.5 ± 0.7	0.015
Final BK biopsy clearance, n (%)	14/26[a] (53.8)	18/22 (81.8)	0.041
Months to biopsy clearance	12.4 ± 12.7	6.2 ± 7.5	0.047
Virological clearance at 3 mo and beyond			
Failed clearance at high level, n (%)	12 (42.9)	4 (18.1)	
Partial SVR (<1000 copies), n (%)	6 (21.4)	4 (18.1)	
SVR (nil detected), n (%)	10 (35.7)	14 (63.3)	NS (0.07)
Quantitative BK level ($\times 10^3$ copies/mL, IQR) partial data	0.12 ± 2.6	0.15 ± 0.37	NS (0.82)
Final BK viremia clearance n (%)	9/27 (33.3)	17 (77.3)	0.044
Time to viremia clearance, mo	29.1 ± 31.8	11.3 ± 10.4	0.015
BK viremia relapse n, (%)	3/11 (27.3)	2/18 (11.1)	NS (0.72)
3 month S. creatinine, μmol/L	235 ± 108	199 ± 102	NS (0.14)
Acute renal dysfunction after immunosuppression reduction, n (%)	22 (78.6%)	13 (59.1%)	NS (0.09)
Treatment with IV methyprednisolone, n (%)	18 (64.3%)	15 (68.2%)	NS (0.78)
Late acute rejection, n (%)	16 (57.1%)	14 (63.6%)	NS (0.95)
Death-censored graft survival from BKVAN diagnosis			
1 y	96.2%	85.0%	
3 y	62.7%	65.6%	NS (0.80)
Patient survival from BKVAN diagnosis			
1 y	92.7%	95.2%	
3 y	88.7%	79.1%	NS (0.69)
Overall graft loss, n (%)	15 (53.6)	6 (27.3)	NS (0.06)
BKVAN as cause of loss, n (%)	10 (35.7)	3 (13.6)	NS (0.08)
Rejection cause of loss, n (%)	5 (17.8)	3 (13.6)	NS (0.69)
Died, n (%)	7 (25%)	4 (18.2%)	NS (0.56)
Follow-up time since BKVAN diagnosis, mo			
Mean (SD)	55.3 ± 46.7	28.5 ± 15.2	NS (0.07)
Median (IQR)	39 (18-75)	27.5 (16-39)	
Study follow-up time (months since transplantation)			
Mean (SD)	98.3 ± 74.7	46.4 ± 27.8	0.003
Median (IQR)	84 (33-138)	44.5 (28-60)	

[a] Repeat biopsy not undertaken in 2 patients.

Mean ± SD.

SVR, sustained viral response.

$P = 0.03$); and with subsequent scarring at 3-month progress biopsy with Banff ct (r = 0.386, $P = 0.01$) and ci (r = 0.342, $P = 0.022$).

Clearance of Viremia and Effect of IVIG Therapy

Quantitative viral load of 1581 ± 4220 × 10^3 copies at diagnosis fell to 1434 ± 7639 × 10^3 midtreatment, and 0.138 ± 0.331 × 10^3 by 3 months. Complete and sustained viral clearance was achieved in 24 patients, partial clearance in 10 (intermittently detectable below 1E + 03 copies/mL), and failed clearance with high-level viremia in 16. By 3 months, complete or partial clearance of viremia was achieved in 18 (81.8%) of 22 with IVIG compared with 16 (57.1%) of 28 by conventional therapy (Upton χ^2 3.378, $P = 0.066$, Table 3, **Table S2, SDC**, http://links.lww.com/TXD/A34).

By univariate Cox regression (**Table S3, SDC,** http://links.lww.com/TXD/A34), overall risk factors for failed viral clearance included late presentation (hazard ratio [HR] [months], 0.951; 95% confidence interval [CI], 0.91-0.99; $P = 0.044$), high initial viral load (HR [$\log_e$ copies/mL], 0.88; 95% CI, 0.79-0.98; $P = 0.024$), shedding of urinary decoy cells (HR, 0.084; 95% CI, 0.012-0.598; $P = 0.013$), greater SV40 scores (HR, 0.424; 95% CI, 0.22-0.82, $P = 0.010$), and acute dysfunction after immunosuppression weaning (HR, 0.40; 95% CI, 0.16-0.73; $P = 0.006$).

Treatment incorporating IVIG more effectively cleared viremia (HR, 3.68; 95% CI, 1.56-8.68; $P = 0.003$), as was tacrolimus conversion to cyclosporine (HR, 4.27; 95% CI, 1.67-10.9; $P = 0.002$) on univariate analysis (Figure 1), whereas sirolimus use was less effective (HR, 0.25; 95% CI, 0.07-0.85; $P = 0.026$). No statistical influence for fluoroquinolone, cidofovir, or initial pulse methylprednisolone therapy was demonstrable (**Table S3, SDC,** http://links.lww.com/TXD/A34). IVIG regime resulted in faster (11.3 ± 10.4 vs 29.1 ± 31.8 months, $P = 0.015$) and more effective (33.3% vs 77.3%, $P = 0.044$) viral blood clearance compared with conventional therapy. A dose-dependent trend of cumulative IVIG dose (HR [gm/kg], 3.24; 95% CI, 0.25-43.0; $P = 0.37$) was insignificant. Multivariate analysis demonstrated independent benefit of IVIG therapy (HR, 6.82; 95% CI, 1.03-45.11; $P = 0.046$) and cyclosporine conversion (HR, 4.264; 95% CI, 1.04-17.44; $P = 0.044$), when adjusted for time posttransplant and SV40 (**Table S4, SDC,** http://links.lww.com/TXD/A34).

IVIG Salvage Treatment Comparisons

IVIG was used in 7 BKVAN cases as salvage treatment after conventional therapy had failed, but was analyzed a priori by final treatment assignment, unfairly disadvantaging the IVIG group. Post hoc analysis of patients receiving IVIG as primary treatment (n = 15, excluding salvage IVIG), demonstrated superior outcomes compared with conventional treatment. Primary IVIG reduced 3-month Banff i scores ($P < 0.05$), SV40T histology ($P < 0.001$) and viremia ($P = 0.002$), improved final biopsy clearance rates to 100% (vs 53.8%, $P < 0.01$), and shortened clearance times (3.6 ± 1.9 vs 12.4 ± 12.7 months, $P < 0.01$), increased SVR (80% vs 35.7%, $P < 0.001$) and viremia clearance times (8.5 ± 9.5 vs 29.1 ± 31.8 months, $P < 0.01$), compared with conventional therapy, respectively (Table 4, **SDC Table 5**). Salvage IVIG used with failing kidneys from uncontrolled BKVAN, with higher 3-month SV40 ($P < 0.05$) and viral loads (511 375 ± 563 026 copies/mL, $P < 0.001$); however, the 3-year graft survival of 57.1% was comparable to conventional (62.7%, Table 4).

Viral clearance from sequential surveillance biopsy was delayed by extensive SV40 staining (HR, 0.21; 95% CI,

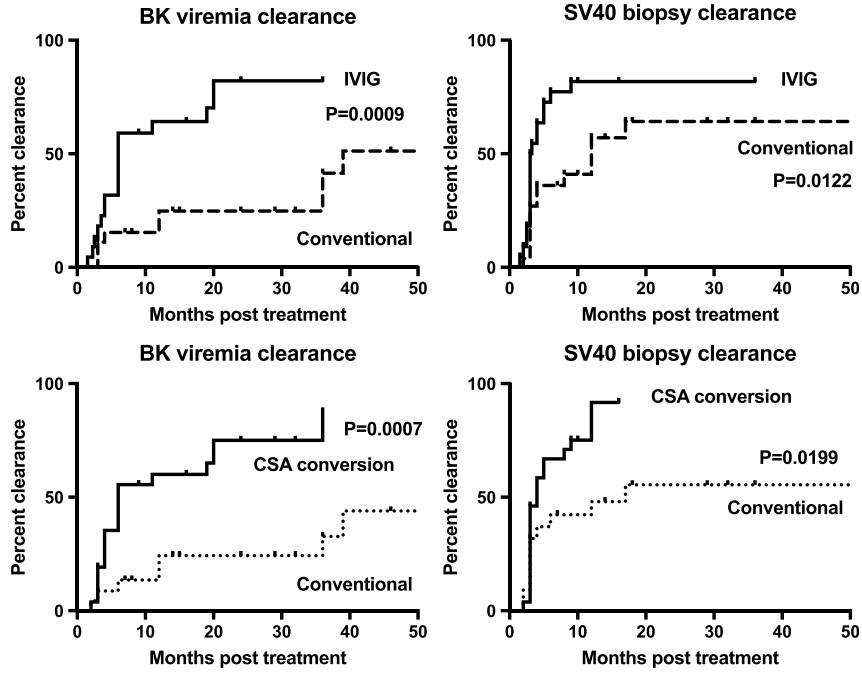

FIGURE 1. Kaplan-Meier curves for clearance of viremia (resolution defined by cutoff as 1000 copies/mL or less as a dichotomous variable, left panels), and biopsy SV40T viral clearance from tissue of repeated biopsy samples (right panels). Upper panels evaluated use of IVIG versus conventional therapy. Lower panels analyze the influence of conversion of tacrolimus to cyclosporine (CSA). *P* values are the log-rank (Mantel-Cox) test statistics for the group differences.

TABLE 4.

Post hoc analysis of treatment groups with IVIG patients subclassified by primary IVIG as initial treatment or late salvage treatment after conventional therapy failed

Factor	Conventional	IVIG	IVIG salvage
Number	28	15	7
Histological clearance			
Last biopsy clearance of virus (%)	14/26 (53.8)	15/15 (100)**	3/7 (42.8)[c]
Months to biopsy clearance (from BKVAN diagnosis)	12.4 ± 12.7	3.6 ± 1.9**	11.9 ± 11.5[b]
Viremia clearance			
Failed clearance with persistent at high-level viremia, n (%)	12 (42.9)	0 (0)	4 (57.1)
Partial SVR, n (%)	6 (21.4)	3 (20.0)	1 (14.2)
SVR, n (%)	10 (35.7)	12 (80.0)**	2 (28.5)[a]
Final BK viremia clearance, n (%)	9/27 (33.3)	14 (93.3)***	3 (42.9)[b]
Time to viremia clearance, mo	29.1 ± 31.8	8.5 ± 9.5**	17.3 ± 10.2[a]
Graft survival			
Death-censored graft survival from BKVAN diagnosis			
1 y	96.2%	92.9%	71.4%
3 y	62.7%	85.1%	57.1%

Mean ± SD.

Primary IVIG versus salvage IVIG: a $P < 0.05$, b $P < 0.01$, c $P < 0.001$.

Conventional versus primary IVIG: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

0.11-0.41; $P < 0.001$), tubular atrophy (HR, 0.48; 95% CI, 0.28-0.82; $P = 0.007$), and interstitial fibrosis (HR, 0.62; 95% CI, 0.38-0.99; $P = 0.047$) on initial diagnostic histopathology (**Table S6, SDC,** http://links.lww.com/TXD/A34). Treatments associated with better histological clearance included IVIG (HR, 2.24; 95% CI, 1.09-4.58; $P = 0.028$) and cyclosporine conversion (HR, 2.25; 95% CI, 1.04-4.86; $P = 0.039$) by univariate analysis, although statistical significance of IVIG (HR, 1.44; 95% CI, 0.61-3.37; P = NS) was lost by multivariate analysis when Banff ct and cyclosporine conversion were included (**Table S7, SDC,** http://links.lww.com/TXD/A34).

Graft Failure and Mortality

Death-censored graft-failure occurred in 21 kidneys (42%) at 32 months (median IQR, 17-55) after diagnosis, due to BKVAN (virus present on final pathology or nephrectomy) in 13 (61.9%) of 21, and acute rejection in 8 (38.1%) of 21 (without SV40 staining or viremia), to 6.3 ± 5.3 years follow up after BKVAN diagnosis. Even when BKVAN was the dominant cause of graft failure, final Banff i scores of 2.21 ± 0.89 indicate unrestrained residual inflammation. The IVIG group experienced fewer overall graft failures (27.3% vs 53.6% for conventional, $P = 0.06$) and less BKVAN losses (13.6% vs 35.7%, $P = 0.08$) at follow-up. By Cox regression, however, graft failure differences were not different by IVIG treatment assignment (HR, 0.88, 95% CI, 0.324-2.39, $P = 0.80$). Overall mortality was 22%, and comparable for IVIG group (HR, 1.28; 95% CI, 0.346-4.76; $P = 0.71$).

DISCUSSION

The destructive and polyoma allograft infection remains a serious threat to the transplanted kidney. Although effective screening strategies reduce incidence, severe BKVAN presenting with active inflammation are difficult to eradicate because of limited therapies with activity against BK virus. Our results demonstrate that adjuvant IVIG when used simultaneously with intensive antiviral treatment including cyclosporine conversion more effectively cleared viremia and histological BKVAN, compared with standard-of-care reduced immunosuppression with cidofovir. A single deep venous thrombosis requiring anticoagulation was the only adverse reaction attributable to IVIG, and other potential reactions, such as headache, allergic reactions, or aseptic meningitis, did not occur with our low-dose regime.

Although further validation of IVIG treatment by randomized trial is needed, the more rapid and complete resolution of BK virus observed is encouraging and agrees with the experience of others. Published data generally comprise small, retrospective single-center observational studies that lack control groups, and some case reports, all using heterogeneous treatment protocols. Nevertheless and despite this variability, IVIG was consistently found to successfully clear BK virus, without excessive acute rejection rates that often penalize immunosuppression reduction. Most BKVAN cases were severe and associated with high-level viremia, active inflammation (category B where graft loss rates of 25-75% are expected) and coexistent allograft dysfunction; and IVIG used as salvage treatment after conventional therapy had failed (as was in 31.8% of IVIG uses in our series).

Sener et al[18] treated 8 patients with BKVAN causing graft dysfunction 11.4 months posttransplant using primary IVIG therapy (2 g/kg divided over 2-5 days) and 50% reduction of immunosuppression, without cidofovir. Viremia was cleared in 50% by 6.5 months, and 88% graft survival at 15.3 months was superior to contemporary published results. Sharma et al[19] reported stabilization of renal dysfunction, clearance of viremia and histological resolution of BKVAN (grade B2) in a 3-year old boy after salvage IVIG treatment (0.6 g/kg × 7 doses), after failed mycophenolate elimination, tacrolimus reduction, and cidofovir. Anyaegbu et al[16] reported clearance of persisting mid-range BK viremia (negative histology) with a single 2 g/kg IVIG dose in 3 pediatric recipients, despite mycophenolate elimination and tacrolimus reduction; and histological resolution of biopsy-proven BKVAN (grade A) in a fourth

child, all with stable allograft function. Vu and colleagues[20] used IVIG (cumulative 1.0 g/kg) to treat 30 BK viremic patients who failed calcineurin inhibitor reduction and mycophenolate replacement with leflunomide (from 53 screened with viremia of 280 transplanted). These comprised 10 histologically proven BKVAN and 20 viremia cases (>10E4 copies with negative pathology) occurring 13.1 ± 5.4 months after transplantation. BK quantitative levels fell rapidly after IVIG treatment, 90% cleared viremia by 1 year, graft survival was 96.7%, and 1 kidney failed from vascular rejection. Dheir et al successfully treated 19 recipients with biopsy-proven BKVAN, viremia and allograft dysfunction (6.8 ± 2.9 months posttransplant); with leflunomide replacement of mycophenolate (68%), cidofovir (32%) and IVIG in 74% (0.5 g/kg daily ×3 days). Two transplants failed from BKVAN, but acute rejection rates were unchanged.

Although these data confirm that IVIG is effective for BKVAN treatment, its mechanism of action is less understood. IVIG is a pooled product containing antibodies to potential pathogens, and by passive immunity reported to clear chronic viral infections from transplanted recients.[12-15] Human IVIG preparations at clinically relevant concentrations contain sufficient neutralizing antibodies against BK virus to inhibit 90% of viral DNA yield, when coincubated with infected human host cells.[24] Some IVIG preparations exceeded 95% viral inhibition at 0.1 μg/mL, without cytotoxicity and impressive therapeutic ratios, easily exceeding cidofovir and lefunomide by in vitro screening.[25] A single 5-g vial of IVIG is estimated to neutralize 9.5E + 09 BK genome equivalents, or 1.9E + 06 genomic copies/ml within 5 L of circulating blood.[24] Pooled IVIG preparations neutralized all BKV genotypes, although high EC50 titres against prevalent genotype Ia fell with the less common genotype IV.[26] Neutralization of virus by antibody was dose-dependent, modestly enhanced by complement, genotype-specific, and achieved without viral aggregation, capsid morphology, elution, or host cell release.[26]

The 150-kDa gammaglobulin major constituent of IVIG, exceeds the upper size limit for glomerular filtration (approximating 70 kDa for podocytes), however, easily diffuses out of the bloodstream into tissues and throughout the extracellular space. The intrarenal distribution of IVIG into interstitial fluid is inferred from ^{131}I radiolabeled immunoglobulin studies of human volunteers. The half-life of intravenous gammaglobulin is 15 to 21 days and gradually exits from the intravascular into the extravascular compartment, reaching equilibration at 5 days, then slower decay kinetics.[27] An apparent mass ratio of extravascular to intravascular exogenously administered gammaglobulin of 1.2 to 1.6 compares with concentration ratios of interstitial fluid to plasma (0.52 and 0.62, for endogenous IgG and albumin, respectively) in normal men.[28] Diffusion of immunoglobulin into the renal interstitial space is likely determined by endothelium type (the fenestrated interendothelial pores exceeding 50 to 60 angstroms characteristic of peritubular capillaries or postcapillary venules exceeds smaller continuous endothelial pores),[29] endothelial uptake and paracellular transport—and influenced by local inflammation. Local interstitial IgG could persist for several weeks after IVIG administration, and neutralizing antibodies against viral capsid protein VP-1 could bind free virus released after lytic tubular cell disruption and limit further viral spread to adjacent cells. The reduced viral SV40 immunohistochemistry scores by 3 months after IVIG treatment support this hypothesis.

Other indirect mechanisms could explain IVIG benefit. IVIG can regulate innate and adaptive immune responses, interacting with dendritic cells, monocytes, and macrophages via its Fcγ receptors. IVIG possesses immunomodulatory properties and can treat refractory T cell–mediated or antibody-mediated rejection.[12,30] IVIG may safely allow reconstitution of an antiviral immune response with reduced immunosuppression, without increasing the risk of acute rejection. Our results showed that inflammation at the diagnosis of BKVAN, and later episodes of acute dysfunction with mononuclear cell infiltration treated with corticosteroids, were both common accompaniments of BKVAN, but comparable between treatment groups. The transcriptional profile of BKVAN and acute rejection are remarkably similar, both expressing excessive proinflammatory and fibrogenic genes.[31] IVIG, by virtue of its immunomodulatory properties, could mitigate the destructive effects of antiviral CD8+ cytotoxic T cells and accompanying noncognate cells which infiltrate the allograft and contribute to "collateral" tubular damage from an exuberant antiviral response.[12] Although greater reduction of mononuclear infiltration occurred in the IVIG group, differences failed significance. Other postulated mechanisms include steric hindrance, conformational change of the bound protein, complement-dependent cytotoxicity, viral agglutination, and phagocytosis.[24]

The strengths of our study include substantial numbers of BKVAN cases, validated by histopathology, detailed granular subject data including multiple transplant pathology and virology during treatment, and use of a control group to allow statistical inferences. The cohort was compliant with STROBE reporting guidelines.[23] One study limitation is its case-controlled cohort study design, which is not randomized. However, study groups were comparable in virtually all BK risk factors at baseline by chance. The only potential confounding factor that required adjustment by multivariate modelling was the greater numbers of patients converted from tacrolimus to cyclosporine in the IVIG group and where multivariate modelling actually demonstrated independent benefit for both strategies. Cyclosporine complexes and inhibits activity of cyclophilin A and NFATc3 (nuclear factor of activated T cells) and suppresses BK viral replication in vitro.[32] We suggest both IVIG and cyclosporine conversion for severe cases of inflammatory BKVAN. Because 31.8% of the IVIG group comprised unresponsive cases of BKVAN used as salvage therapy after conventional therapy failed, the benefit of IVIG is underestimated, as indicated by the sensitivity analysis.

If the effectiveness of IVIG is established by future trials, its place in management algorithm of BKVN protocols will then need clarification, with optimal dose (low vs high total dose) and frequency (single-larger vs frequent-smaller doses), and duration of therapy. We propose IVIG as adjuvant therapy, added synchronously with intensive approach using a combination of cyclosporine, leflunomide, cidofovir, and ciprofloxacin, rather than substitution of a single component of a strategy. Because of increased rejection risks with reduced immunosuppression for BKVAN, we suggest adjuvant IVIG combination as initial primary therapy, where histology demonstrates interstitial inflammation (pattern associated with substantial graft loss), and for those patients of high

immunological risk, rather than fail conventional therapy with graft damage and use for late salvage treatment.

In conclusion, combination treatment incorporating IVIG was more effective in clearing viral expression in patients with BKVAN, compared with conventional standard-of-care treatment. Prospected multicenter randomized trials are needed for validation of its efficacy, and indications for use and optimal incorporation into current management algorithms need to be established.

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