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Late-Onset Hemorrhagic Cystitis in Children after Hematopoietic Stem Cell Transplantation for Thalassemia and Sickle Cell Anemia: A Prospective Evaluation of Polyoma (BK) Virus Infection and Treatment with Cidofovir

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Little is known about late-onset hemorrhagic cystitis (HC) in children, its relationship to BK virus, and treatment with cidofovir (CDV) following hematopoietic stem cell transplantation (HSCT). We prospectively investigated BK virus reactivation in children who underwent HSCT from a matched related donor for thalassemia or sickle cell anemia following busulfan-cyclophosphamide–based conditioning regimens and analyzed risk factors for development of HC and its treatment with CDV. Grade 2-4 HC occurred in 30 patients with a cumulative incidence of 26% (95% confidence interval [CI] = 18%-34%). The cumulative incidences of BK viruria and viremia were 81% (95% CI = 69%-89%) and 28% (95% CI = 18%-40%), respectively. Multivariate analysis revealed that use of antithymocyte globulin (ATG) (hazard ratio [HR] = 10.5; P = .001), peak BK viruria >100,000 copies/mL (HR = 6.2; P = .004), and grade II-IV acute graft-versus-host disease (HR = 5.3; P = .007) were predictive factors for HC. Nineteen patients with HC were given CDV at 1.5 mg/kg/day 3 times a week, or 5 mg/kg/week. The median duration of therapy was 27 days (range, 21-180 days), and a median of 9 doses were given (range, 6-22). All patients had a complete clinical response (CCR), and 69% had a microbiological response at 4 weeks. Eleven patients with BK virus–related HC receiving supportive care also had CCR. The median duration of HC in these patients was similar to that in patients treated with CDV. None of the patients with HC cleared BK viruria when CCR was achieved. We conclude that late-onset HC is more prevalent in children with sustained high BK viruria who are treated with ATG or who develop graft-versus-host disease. Randomized clinical trials are urgently needed to better define the role of CDV in treating BK virus–related HC.

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KEY WORDS: Hemorrhagic cystitis, BK virus, Cidofovir, Hemoglobinopathies, Stem cell transplantation

INTRODUCTION

Hemorrhagic cystitis (HC) is one of the major complications of hematopoietic stem cell transplantation (HSCT), causing significant morbidity and prolonged hospitalization. The urologic manifestations vary from asymptomatic hematuria to bladder pain with irritative voiding and/or severe bladder hemorrhage leading to clot retention and renal failure. The overall incidence of HC has been reported to vary between 7% and 68% [1-3]. Early-onset HC usually occurs during or immediately after high-dose cyclophosphamide (Cy) administration as part of the conditioning regimen, whereas late-onset HC develops beyond 1 week after transplantation, usually related to an infectious cause. The reported incidence of

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late-onset HC varies between 10% and 22% [4-6]. BK virus (BKV) is most frequently associated with late-onset HC [4-8], although adenovirus- and cytomegalovirus (CMV)-associated HC also occur [9,10].

BKV is a polyomavirus belonging to the Polyomaviridae family. Primary BKV infection occurs during childhood, resulting in almost-universal seropositivity [11]. Following primary infection, the virus remains latent in the renourinary tract as the epidemiologically most relevant latency site, as well as in the lung, liver, brain, lymphocytes, and probably other tissues [12]. Reported seroprevalence rates in adults range between 60% and 90% [12,13]. Initial studies identified a qualitative association between BKV and HC [2,7,14]. Recently introduced quantitative polymerase chain reaction (PCR) techniques allow the determination of BKV DNA load in urine and/or blood samples in HSCT recipients; most studies to date have confirmed a quantitative association between BK viruria and HC, with patients with HC having higher viral loads in the urine and blood [15-18]. About 50% of HSCT recipients with persistent BK viruria do not develop HC, however, indicating that other factors contribute to this complication [2,16]. Most of these studies were performed in heterogeneous adult populations with hematologic malignancies to date, the role of BKV in the development of HC in children, especially those with nonmalignant diseases, has not been evaluated. Furthermore most studies detected the virus at the time of cystitis, therefore not allowing estimation of the relationship between BK reactivation and HC.

Treatment of HC varies depending on severity. The current standard of care for HC is symptomatic and includes analgesia, hyperhydration, forced diuresis, and bladder irrigation to prevent clot formation and renal failure. When conservative measures fail or bleeding is intractable and life-threatening, surgical intervention is considered. Various topical agents, including alum, formalin, prostaglandin E1, and hyperbaric oxygen, have been used with controversial results, because of the absence of controlled studies. To date, no antiviral drug with proven efficacy against BK replication has been approved for use. Cidofovir (CDV) is a cytosine derivative of an acyclic nucleoside-phosphonate analogue with broad-spectrum activity against many DNA viruses, including CMV, adenoviruses, and polyomaviruses [19]. CDV has been used to treat BKV–related nephropathy in limited renal transplantation recipients [20] and bone marrow (BM) transplantation recipients [21,22], with variable results.

In the present study, we tested the hypothesis that BKV reactivation alone is not sufficient to cause late-onset HC, and the presence of coexisting risk factors is required. To investigate this hypothesis, we prospectively monitored BKV in urine and blood through quantitative PCR assays in a homogeneous patient population treated with a busulfan-Cy (BuCy)- based conditioning regimen. We also studied the efficacy of CDV for treatment of HC. To the best of our knowledge, this is the first study to evaluate the incidence of BKV, its potential role in the development of HC, risk factors for HC, and the efficacy of CDV therapy in children undergoing HSCT for thalassemia and sickle cell anemia (SCA).

PATIENTS AND METHODS

Patients

All patients who had thalassemia or SCA and underwent HSCT from an HLA-matched family donor were eligible for this study. Patients aged >17 years and who received an HLA-mismatched transplant were excluded from the analysis. The institutional review board approved the treatment protocol, and all parents or patients provided written informed consent, in accordance with the Declaration of Helsinki.

Between July 2004, and March 2009, 117 patients with thalassemia (n = 107) and SCA (n = 10) who received a BM graft from an HLA-matched family donor were enrolled in this study. Seven patients underwent a second transplantation from the same donor. All but 4 patients received unmanipulated BM as the stem cell source; those 4 patients received unmanipulated peripheral blood stem cells. The median patient age was 8 years (range, 1.7-17 years); the median donor age was 9.9 years (range, 1.8-53 years). Twenty-nine patients had undergone splenectomy before HSCT. The vast majority of the patients had severe iron overload, and most patients demonstrated some degree of liver fibrosis at the time of transplantation. On the basis of 3 risk factors (hepatomegaly >3 cm, presence of liver fibrosis, and irregular chelation history), patients with thalassemia are categorized into 3 risk classes. Class 1 patients have no risk factors, class 2 patients have 1 or 2 risk factors, and class 3 patients have all 3 risk factors. These risk classes are not applicable to patients with SCA. Patient and transplant characteristics are summarized in Table 1.

Conditioning Regimens and GVHD Prophylaxis

Conditioning regimens used were all BuCy-based (Table 1). Based on our experience, very young patients (aged <4 years), patients who receive a BM graft from a phenotypically identical donor, and patients with SCA have an increased rejection rate when conditioned with a BuCy-based regimen alone. Therefore, we added thiotepa (TT) or antithymocyte globulin (ATG; Thymoglobulin; Genzyme, Lyon, France) to the conditioning regimen of these subgroups of patients, in an attempt to decrease the rejection rate. Fifty-nine patients (50%) received TT and

Table 1. Patient and Transplant Characteristics

Variable	
Number of patients	117
Sex, M/F, n	67/50
Age, years, median (range)	8 (1.7-17)
Diagnosis, n	
Thalassemia	107
Sickle cell anemia	10
Risk class, n	
Class 1/class 2/class 3	19/38/50
Not applicable	10
Red blood cell units received before transplantation, median (range)	79 (2-307)
Serum ferritin, ng/mL, median (range)	1974 (279-10,222)
Liver iron, mg/g dry weight, median (range)	13 (0.6-48)
Aspartate aminotransferase, IU/L, median (range)	32 (13-216)
Alanine aminotransferase, IU/L, median (range)	35 (3-224)
Bilirubin, mg/dL, median (range)	1.1 (0.4-5.5)
Splenectomy, n	29
Liver fibrosis score (Ishak et al. [23] staging score 0-6), median (range)	1 (0-5)
Not evaluable, n	8
HLA-identical siblings, n	106
HLA phenotypically identical parents or relatives, n	11
ABO blood group compatibility, identical/mismatched, n	75/42
CMV serostatus, positive/negative, n	100/17
Stem cell source, n	
Bone marrow	113
Peripheral blood	4
Conditioning regimen, n	
Bu/Busilvex/Cy 200	23
Bu/Busilvex/TT 10/Cy 200	25
Bu/Busilvex/Cy 160 preceded by HU, AZA, and Flu	28
Bu/Busilvex/TT 10/Cy 160 preceded by HU, AZA, and Flu	15
Bu/Busilvex/TT 10/Cy 200/ATG 10 preceded by HU, AZA, and Flu	17
Bu/Busilvex/Cy 200/ATG 10	9
GVHD prophylaxis, n	
CsA+ methylprednisolone 0.5 mg/kg + short MTX	56
CsA + methylprednisolone 0.5 mg/kg + Cy 7.5 mg/kg (d +1) + short MTX	61
Nucleated cell dose, $\times 10^8$/kg, median (range)	4.6 (1.3-20.8)
$CD34^+$ cell dose, $\times 10^6$/kg, median (range)	7.1 (0.8-35)
$CD3^+$ cell dose, $\times 10^6$/kg, median (range)	53 (3.8-330)

MTX indicates methotrexate; CsA, cyclosporine A; Bu, busulfan; TT, thiotepa; HU, hydroxyurea; AZA, azathioprine; Flu, fludarabine.

26 patients (22%) received ATG. ATG was given at a dose of 2-2.5 mg/kg/day for 4 or 5 days before transplantation. Sixty-nine patients (59%) with advanced thalassemia (risk class 3) or receiving a transplant from a phenotypically identical family donor or a second transplant received cytoreduction/immunosuppression with hydroxyurea 30 mg/kg and azathioprine 3 mg/kg daily from day -45 pretransplantation, and fludarabine 20-30 mg/m^2 from day -16 through day -12 before the conditioning regimen. Starting in June 2006, all patients received targeted i.v. Busilvex (Pierre Fabre Medicament, Boulogne Billancourt, France; target area under the curve, 900-1350 mmol/min) instead of oral Bu. GVHD prophylaxis consisted of cyclosporine, methylprednisolone, and a short course of methotrexate. Diagnosis of acute GVHD (aGVHD) and chronic GVHD was based on standard clinical criteria, with histopathological confirmation when possible. Patients received a stem cell infusion 36 hours after the last dose of Cy.

Prophylaxis Against HC

Prophylaxis against HC consisted of sodium 2-mercaptoethane sulfonate administration with 120% of the daily Cy dose in 6 divided doses, starting immediately before each dose of Cy. Patients received i.v. hydration at 3000 mL/m^2/24 hours beginning 14 hours before Cy administration and continuing until 24 hours after the last dose of Cy, along with forced diuresis and alkalization of the urine. During Cy administration, a Foley catheter was placed to ensure immediate drainage of bladder and hence decrease bladder exposure to acrolein in all patients.

Diagnosis of HC

Patients who had early HC (occurring within 1 week post-HSCT) were excluded from the analysis. Only patients with late-onset HC (occurring beyond 1 week post-HSCT) were analyzed in this study. The maximum severity of HC was graded according to published criteria: grade 0, no hematuria; grade 1, microscopic hematuria; grade 2, macroscopic hematuria; grade 3 macroscopic hematuria with clots; grade 4, macroscopic hematuria with clots and impaired renal function secondary to urinary tract obstruction. A diagnosis of HC required the presence of clinically significant hematuria (grade 2-4) along with dysuria or lower abdominal pain (or both). The worst clinical presentation was considered the maximum grade.

Sampling of Specimens

Midstream urine and blood samples for BKV and adenovirus were collected immediately before initiation of the conditioning regimen, and then weekly thereafter until at least 100 days post-HSCT. Microscopic urinalysis and examination for microscopic or macroscopic hematuria were performed on all midstream urine specimens 3 times weekly during hospitalization and twice weekly after discharge. When HC developed, urine samples also were investigated for CMV. A total of 1267 urine and blood samples were obtained from 64 patients (10-50 samples/patient).

PCR for BKV DNA Quantification in Serum and Urine Samples

Before August 2006, qualitative PCR for BK viruria was performed on urine samples in 15 patients who developed HC. Between August 2006, and August 2009, PCR for BK DNA detection was performed in plasma and urine samples from 64 patients. Urine and blood samples were collected on the same day. Qualitative PCR was performed in the first 9 patients,

and both qualitative and quantitative PCR were performed in the subsequent 55 patients. DNA from plasma and urine samples was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). BKV DNA quantification was performed by real-time TaqMan PCR using a commercial kit (BKV Q-PCR Alert Kit; Nanogen Advanced Diagnostics, San Diego, CA) for the detection of the target viral gene encoding for large T-antigen of the BK virus with the 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA). BK viral load sensitivity and specificity were 100% and >95%, respectively.

Treatment of HC

The standard of care consisted of analgesia, hyperhydration, forced diuresis, and platelet transfusion to maintain a platelet count at $\geq 50 \times 10^9$/L. Eleven patients who developed HC before the prospective trial of CDV received standard supportive treatment. Nineteen patients with HC were given CDV at a dose of 1.5 mg/kg/day 3 times a week or 5 mg/kg/day once a week. Probenecid, 1.25 g/m^2 by mouth, was given 3 hours before and 1 hour and 8 hours after CDV administration. Patients received i.v. hydration at 3 times maintenance level, initiated 1 hour before and continuing until 1 hour after the completion of the drug infusion, followed by hydration at 2 times maintenance level for an additional 2 hours after CDV administration.

Supportive Care

Patients were given prophylactic broad-spectrum antibiotics and antifungal drugs (amphotericin B preparations) until the neutrophil level exceeded 1.0×10^9/L. Patients also received oral nonabsorbable antifungal nystatin and paromomycin for gut decontamination from the start of conditioning regimen. Acyclovir was administered for herpes virus, and trimethoprim/sulfamethoxazole was given for *Pneumocystis jiroveci* prophylaxis. All patients received i.v. immunoglobulin at a dose of 250-500 mg/kg once weekly until day 100 post-HSCT for infection prophylaxis. All blood products were irradiated to 2500 cGy. CMV antigenemia monitoring was done twice weekly at hematologic recovery. Chimerism analysis was performed by PCR-based analysis of short tandem repeats (Profiler Plus; Applera, Foster city CA). In sex-mismatched donor–recipient pairs, 2-color fluorescein in situ hybridization also was performed according to standard procedures. Grade II or greater aGVHD was treated with methylprednisolone 1-4 mg/kg/day.

Statistical Analysis

Data on pretransplantation patient characteristics, transplant complications, and outcomes were prospectively collected. The primary endpoint was grade 2-4 HC. Secondary endpoints were treatment-related mortality (TRM) and survival. Probabilities of HC, TRM, and aGVHD and chronic GVHD were calculated using cumulative incidence curves to accommodate competing risks [24]. For HC, graft failure and death without HC were considered as competing events. Probabilities of survival and disease-free survival were calculated using the Kaplan-Meier estimator [25]. The effects of patient-, donor-, disease-, and treatment-related variables on development of HC were evaluated using cumulative incidence curves and compared with the Gray test. Prognostic factors for the occurrence of HC found to be significant or nearly significant by univariate analysis were evaluated using the proportional hazards model for competing-risk regression analysis [26]. The variables included diagnosis, patient age, donor type, conditioning regimen, use of ATG in the conditioning regimen, donor–recipient CMV serology, donor–recipient sex match, Cy dose, seasonality, risk class, number of red blood cell units received before transplantation, splenectomy, degree of hepatomegaly, serum ferritin level, liver iron concentration, second transplantation, early HC because of drug toxicity, speed of neutrophil recovery, platelet engraftment, presence of aGVHD, BK viruria, BK viremia, peak BK viruria, and viremia. All P values were 2-sided, with $P < .05$ indicating statistical significance. Statistical analyses were performed using StatView 5 (SAS Institute, Cary, NC) and R version 2.9.1 [27].

RESULTS

A total of 99 patients experienced sustained engraftment. Graft failure/rejection occurred in 8 patients (n = 2 with primary graft failure; n = 6 with secondary graft failure). The median time to neutrophil recovery (defined as the first of 3 consecutive days on which the neutrophil count exceeded 0.5×10^9/L) was 20 days (range, 12-43 days), and the median time to a platelet recovery (defined as the first of 7 consecutive days that the platelet count exceeded 20×10^9/L without platelet transfusion) was 23 days (range, 8-163 days). Forty patients (34%) had grade II-IV aGVHD.

Clinical Course and Incidence of HC

Thirty of 117 patients developed clinically significant (grade 2-4) late-onset HC within 1 year after transplantation. The median time to onset of HC was 38 days (range, 13-114 days), with only 1 patient presenting after 100 days. In 1 patient, HC occurred before platelet engraftment, and in the remaining 29 patients, HC developed more than 26 days after transplantation. The median platelet count at the onset of HC was 81×10^9/L (range, 2-274 $\times 10^9$/L). Four patients had grade 1 HC (without urinary symptoms), 24 patients had grade 2 HC, 2 patients had grade 3 HC, and 4 patients had grade 4 HC. Sixteen of the

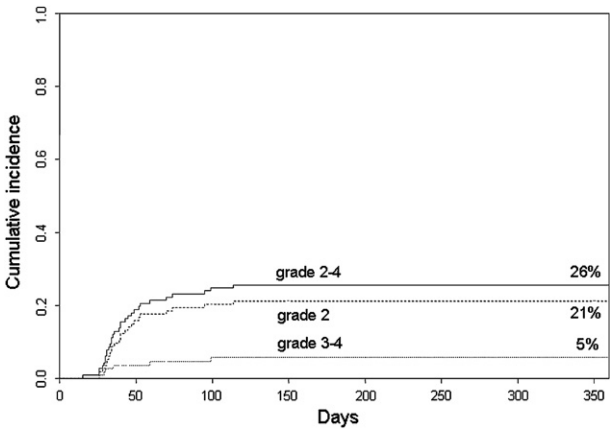


Figure 1. Cumulative incidence of hemorrhagic cystitis.

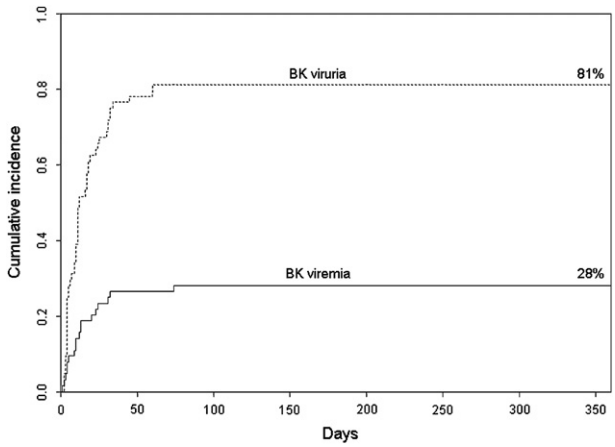


Figure 2. Cumulative incidence of BK reactivation in urine and blood.

30 patients with grade 2-4 HC had grade II-IV aGVHD. In all but 2 of these patients, HC development preceded GVHD, by a median of 23 days (range, 7-96 days). Ultrasound examination revealed bilateral grade 3-4 hydronephrosis in the 4 patients with grade 4 HC. All of these patients had impaired renal function and moderate to severe flank pain poorly controlled with repeated analgesic drugs, necessitating ureteral stent placement. A contrast computed tomography scan in 1 of these patients, who had severe intermittent abdominal and flank pain, revealed bilateral grade 4 hydroureteronephrosis and left-sided ureteral stenosis. This patient underwent retrograde stent placement on the right side and antegrade stent placement through percutaneous nephrostomy because of high-grade obstruction in the left ureter. In all 4 patients, the blood creatinine level dropped to baseline level by the following day, with complete resolution of flank pain. The stents were removed 2-6 months later with no future impairment of renal function in all 4 patients. The cumulative incidence of grade 2-4 HC was 26% (95% confidence interval [CI] = 18%-34%), including 21% (95% CI =13%-28%) for grade 2 HC and 5% (95% CI = 2%-10%) for grade 3-4 HC (Figure 1). Early HC occurred in 6 patients, with a cumulative incidence of 5% (95% CI = 2%-10%). All these patients had grade 2 HC with minor urinary symptoms that resolved within a few days with supportive care only, and none subsequently developed late-onset HC.

BKViruria and BKViremia

Nine of the 64 patients prospectively monitored underwent qualitative PCR, and the remaining 55 patients underwent both qualitative and quantitative PCR for BKV DNA. Sixty patients (94%) had at least 1 positive sample and 52 (81%) had 2 or more positive samples for BK viruria. Thirty-four patients (53%) had at least 1 positive sample and 18 (28%) had 2 or more positive samples for BK viremia. Viruria and viremia were defined as sustained in the presence of 2 or more positive urine and plasma samples, and as transient otherwise. In 14 patients, the first BK viruria occurred before HSCT, during conditioning; 9 of these patients were in risk class 3 and had received preconditioning chemotherapy with hydroxyurea, azathioprine, and fludarabine. The median times to detection of sustained BK viruria and BK viremia were 8.5 days (range, 0-60 days) and 11 days (range, 0-74 days), respectively. BK viremia never occurred in the absence of viruria and almost always followed BK viruria. The median interval from the onset of viruria to the onset of viremia was 19 days (range, 0-69 days). After the onset of viruria, levels of BK viral DNA in the urine increased progressively, with a median interval from onset to peak level of 14 days (range, 0-146 days). The estimated incidences of sustained BK viruria and BK viremia were 81% (95% CI = 69%-89%) and 28% (95% CI = 18%-40%), respectively (Figure 2). The median numbers of peak viral copies in the urine and in the blood were 21,363 copies/mL (range, 556-55 $\times$ 10^6 copies/mL) and 578 copies/mL (range, 556-16,480 copies/mL), respectively. The mean number of peak viral copies in the urine was higher in patients who received ATG compard with those who did not (44 $\times$ 10^6 copies/mL vs 17 $\times$ 10^6 copies/mL; P = .017, Mann-Whitney test). All 30 patients with grade 2-4 HC had BK viruria. Among the 52 patients with sustained BK viruria, 15 (29%) developed HC. The median time from peak BK viruria to development of HC was 15 days (range, 8-75 days). Thirteen of 26 patients (50%) who had peak BK viruria >100,000 copies/mL developed HC, compared with 2 of 27 patients (7%) with peak BK viruria <100,000 copies/mL. However, 10 patients with peak BK viruria between 6 $\times$ 10^6 and >55 $\times$ 10^6 copies/mL at 2 or more time points did not develop HC. The 4 patients with grade 1 HC had BK viruria, whereas all of the other patients without HC but with BK viruria had no microscopic hematuria.

We also investigated coinfection with other viruses known to lead to HC in HSCT recipients. In th 30 patents with HC, 2 patients had CMV and 1 patient had adenovirus. The 2 patients who had CMV in the urine also had simultaneous CMV reactivation in the blood, hindering assessment of its contribution to HC. The patient with simultaneous adenovirus in the urine had persistent BK viruria of $>55 \times 10^6$ copies/mL. The adenovirus was not isolated from any other site (ie, blood, feces, saliva) in this patient. CMV reactivation detected by antigenemia occurred in 87% of the patients who developed HC. Urine cultures obtained from patients with HC were negative for bacterial infection.

Risk Factors

Univariate analysis revealed a significantly higher prevalence of HC in patients who received ATG in the conditioning regimen (Figure 3; $P = .00001$), had peak BK viruria >100,000 copies (Figure 4; $P = .0009$), or developed grade II-IV aGVHD (Figure 5; $P = .014$). In addition, patients aged >8 years also had a higher incidence of HC, with a trend toward significance ($P = .056$). None of the other variables listed above was associated with the development of HC. Multivariate analysis using the proportional hazards model for competing-risk analysis confirmed the prognostic importance of ATG (hazard ratio [HR] = 10.5; $P = .001$), peak BK viruria (HR = 6.2; $P = .004$), and aGVHD (HR = 5.3; $P = .007$), but not of patient age, for the development of HC (Table 2). The cumulative incidence of HC in patients who had all 3 adverse risk factors was 88% (95% CI = 17%-98%), compared with 64% (95% CI = 27%- 86%), 30% (95% CI = 12%-51%), and 0% in those with 2, 1, or none of these risk factors, respectively (Figure 6).

CDV Therapy

Two schedules of CDV therapy were used. At the beginning of this prospective trial, in the first 10 patients we administered CDV 1.5 mg/kg/day 3 times a week for at least 9 doses, because of toxicity concerns. Once we saw that these patients exhibited no drug-related nephrotoxicity, we gave the next 9 patients CDV at a dose of 5 mg/kg/day once a week for the first 2 weeks, then once a week every other week thereafter. Patients with grade 3-4 HC were given 5 mg/kg/day of CDV weekly for 4 consecutive weeks, followed by biweekly administration. CDV was given until complete resolution of HC. In patients with grade 3-4 HC, maintenance therapy with CDV was continued until a >2 log decrease in urine viral load was detected. Immunosuppressive prophylaxis against GVHD was not modified in these patients. Patients with concomitant GVHD also were treated with steroids. The median time from onset of HC to start of CDV therapy was 2 days (range, 0-34 days). In all but 4 patients, CDV therapy was started as soon as the patient manifested urologic symptoms.

The median duration of HC was 19 days (range, 10-53 days). The median duration of CDV therapy was 27 days (range, 21-180 days), and a median of 9 doses were given (range, 6-22 doses). All patients treated with CDV demonstrated a complete clinical

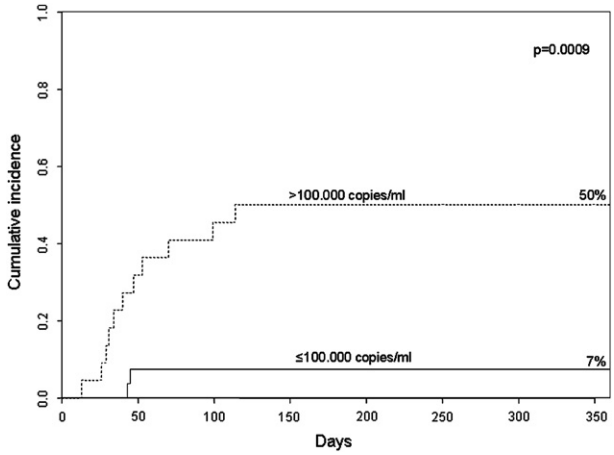


Figure 4. Cumulative incidence of hemorrhagic cystitis according to BK viral load in the urine.

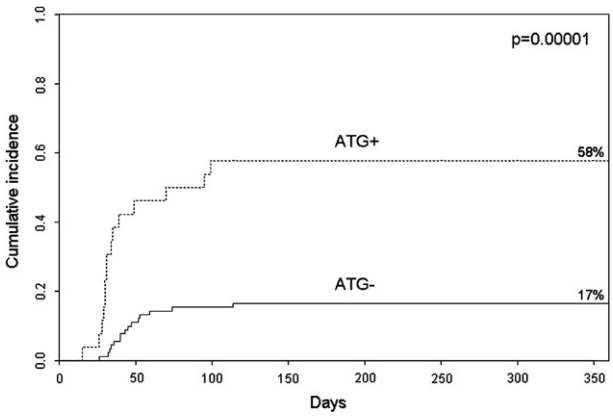
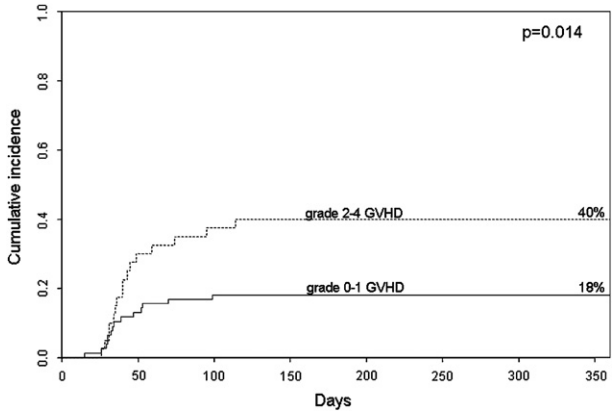


Figure 3. Cumulative incidence of hemorrhagic cystitis according to use of ATG in the conditioning regimen.



Fugure 5. Cumulative incidence of hemorrhagic cystitis according to grade II-IV acute GVHD.

Table 2. Proportional Hazards Model for Competing-Risk Regression Analysis

Variable	HR	95% CI	P
ATG in the conditioning regimen	10.5	2.5-43	.001
Peak BK viruria	6.2	1.8-21	.004
GVHD	5.3	1.6-18	.007

ATG indicates antithymocyte globulin; GVHD, graft-versus-host disease.

response. Among the 13 evaluable patients, microbiological response (defined as a 1-2 log decrease in viral load) occurred in 5 patients (39%) at 2 weeks and in 9 patients (69%) at 4 weeks. However, none of CDV-treated patients cleared BK viruria when a complete clinical response was achieved. The median time from clinical response to clearance of BK viruria was 74 days (range, 24-176 days). No patient experienced HC recurrence after discontinuation of CDV. No difference in BK virus clearance was noted between the 2 different dosing schedules. CDV therapy was well tolerated, with no significant nephrotoxicity (data not shown). Despite the adminstration of concurrent nephrotoxic drugs (eg, cyclosporine, Prograf, amphotericin B), no patient exhibited a significantly increased serum creatinine level or discontinued therapy. Four patients (21%) had transient mild proteinuria the day after CDV administration. None of CDV-treated patients experienced bone marrow suppression resulting in neutropenia. There was no correlation between CDV dosage and renal toxicity. Ten patients with grade 2 HC and 1 patient with grade 3 HC before this prospective trial of CDV were treated with supportive care. The median duration of HC in these patients was 11 days (range, 9-60 days), not significantly different from that in the patients treated with CDV. The CDV-treated patents included more cases of grade 3-4 HC, however.

Survival and TRM

With a median follow-up in survivors of 35 months (range, 5-61 months), 107 patients were alive, and 100 of them were disease-free. One-year overall and disease-free survival were 92% (95% CI = 86%-96%) and 85% (95% CI = 78%-90%), respectively. No patient died from HC. Three patients died within the first 100 days post-HSCT, and 7 patients died within 1 year post-HSCT. HC had no significant impact on survival. Causes of death were GVHD (n = 6), infection (n = 3), and posttransplantation splenectomy (n = 1).

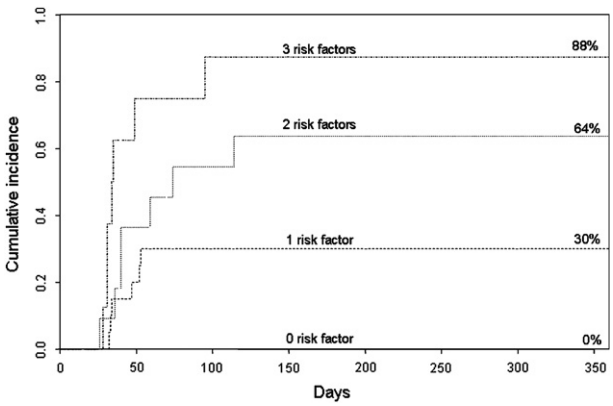


Figure 6. Cumulative incidence of hemorrhagic cystitis according to risk factors.

DISCUSSION

HC is a major complication in patients undergoing HSCT. Current potent immunosuppressive treatment protocols have significantly improved graft survival and GVHD control in HSCT recipients, likely resulting in an increased rate of infection in these patients, especially opportunistic viral reactivation. As opposed to early-onset HC, which occurs during the conditioning regimen or within the first week of its completion due to drug toxicity, later HC is usually associated with viral infections and frequently is more severe and occasionally fatal [28,29]. The incidence of late-onset HC in our patients was comparable to that reported previously in other pediatric patients [6,21].

There is accumulating evidence in the literature supporting a strong association of BKV with urinary tract pathology due to the urotropic nature of the virus [2,7,11,30]. Further elucidation regarding the role of BK virus infection in HC development in stem cell transplant recipients is clearly needed. The association between BKV and HC in BM transplantation recipients was first reported by Arthur et al. [7]. However, most of the previous studies evaluating risk factors for HC and its relationship to BKV were performed in either small numbers of pediatric patients or in heterogeneous populations and involved different stem cell sources and preparative regimens [6,21]. We prospectively studied BK viruria and viremia in a homogeneous pediatric patient population treated with a BuCy-based conditioning regimen, which allowed us to define the role of BKV and risk factors for development of late-onset HC in this context. This is the first study to evaluate the incidence of the BKV and its relationship to HC in children with thalassemia and SCA. We found that children with hemoglobinopathies have a high incidence of BKV reactivation following HSCT. The cumulative incidences of sustained BKV reactivation in the urine and in the blood were 81% and 28%, respectively. BKV reactivation is associated with a failure of T cell immunity in HSCT recipients resulting from both the conditioning regimen and immunosuppressive prophylaxis against GVHD. In the present study, we investigated the relationship between HC and CD3, CD4, and CD8 lymphocyte recovery at 30 and 60 days post-HSCT. We found no statistically significant

difference between the 2 groups of patients (data not shown), suggesting that it is the number of BK virus–specific cytotoxic T lymphocytes, not the absolute number of lymphocytes, that plays an important role in controlling BKV reactivation [31].

Our findings demonstrate that despite a high BKV reactivation rate, especially in the urine, HC occurred in only 29% of patients with sustained BK viruria. This indicates that BKV infection is not specific or universal for HC and supports a more contributory role of the virus in predisposed immunocompromised HSCT recipients. In fact, 10 of our patients with persistently higher BK viruria (between 6×10^6 and $>55 \times 10^6$ copies/mL) never developed HC, indicating that late-onset HC is a multifactorial disease that requires other cofactors for its development. These data are in line with some previously published results [15,16,21]. Furthermore, we found that high, but not persistent, BK viral load in the urine is a risk factor for development of HC, also in agreement with previous studies [5,6,13,16,32]. Interestingly, BK viremia never occurred in the absence of BK viruria and almost also followed BK viruria. Neither persistent nor peak BK viremia was associated with HC in our patients. This contrasts with data reported by Erard et al. [18], who showed that BK viremia detected during disease was independently associated with HC and that the higher the viral load, the greater the risk of HC.

In both renal transplant and HSCT recipients, more intense immunosuppression may increase BKV replication in the urinary tract, leading to increased local inflammation and subsequent development of HC. In the present study, the use of Thymoglobulin in the conditioning regimen was strongly associated with HC in both univariate and multivariate analyses (HR = 10.5; $P = .001$). Thymoglobulin is known to be more potent than other ATG preparations in preventing organ rejection [33]. An association between high Thymoglobulin dose and increased infections has been reported previously [34]. Increased immunosuppression with Thymoglobulin increased viral replication with higher viral loads in the urinary tract in our patients; mean peak BK viruria was significantly higher in those receiving Thymoglobulin compared with those not receiving Thymoglobulin. Thus, we recommend careful viral monitoring in patients treated with Thymoglobulin.

It has been suggested that an association between GVHD and HC might exist, or that immunosuppressive therapies used to treat GVHD increase the risk of opportunistic infections, which subsequently cause HC [32,35]. Nevertheless, some studies have failed to show any correlation between GVHD and HC [2,36]. In the present study, we found a higher rate of HC in patients with grade II-IV aGVHD compared with those with grade 0-I GVHD (HR = 5.3; $P = .007$), suggesting that GVHD and/or its treatment contribute to the increased risk of HC.

Four of our patients developed hydronephrosis with impaired renal function. To the best of our knowledge, this is the first case series of ureteral obstruction because of BK virus–related HC and resultant ureteritis in pediatric HSCT recipients successfully resolved with stent placement. A similar case has been reported in a young adult HSCT recipient [37]. We stress the importance of ultrasonographic renal monitoring in patients with HC for timely diagnosis of hydronephrosis, followed by ureteral stent placement when necessary to prevent the development of obstructive renal failure.

We also investigated coinfection with other viruses, such as adenovirus and CMV, known to lead to HC in HSCT recipients [9,10]. Urine and blood samples also were prospectively evaluated by adenovirus-specific PCR. Two of the 64 patients (3%) had adenovirus in the urine, which was the only site in which the virus was isolated. One of these patients had HC along with persistently higher BK viruria. Although it is unlikely, we cannot rule out a possible role of adenovirus in the development of HC in this patient.

Treatment of late-onset HC remains a major therapeutic challenge. Early and close collaboration between the transplantation physician and urologist is essential for a successful outcome. There are no antiviral drugs with proven efficacy for treating BKV–related HC. CDV is currently the most selective antipolyomavirus agent [19], although the mechanism by which CDV mediates is not clear, because unlike herpesviruses, polyomaviruses do not have viral-encoded DNA polymerase. The experience with using CDV to treat BK V infection comes from the renal transplantation setting, and nephrotoxicity has limited its use in HSCT recipients, who typically are exposed to other nephrotoxic drugs. To date, no prospective clinical trial has evaluated the use of CDV in children and adolescents with BK virus–related late-onset HC. In a recent prospective case series, 22 pediatric HSCT recipients with BKV/JC virus or adenovirus-associated HC were given CDV 5 mg/kg twice weekly initially, then fortnightly, which resulted in complete eradication of the virus from the blood, but not from urine [21]. No nephrotoxicity was observed in these patients. Low-dose CDV (1 mg/kg/week) without probenecid was given to adults [22] and a small number of pediatric patients [38] with the aim of avoiding nephrotoxicity and achieving higher urinary concentrations, with variable results. In the present study, we prospectively treated 19 patients with CDV. These patients' immunosuppressive medications were not changed, because of concerns about GVHD. Patients with HC and simultaneous aGVHD were treated with steroids. All patients recovered from HC; however, none of them had cleared the BKV from the urine by the time of complete resolution of HC. Although BK viruria persisted, no patient experienced recurrence of HC after discontinuation of

CDV. CDV therapy was well tolerated, with no dose-limiting nephrotoxicity or BM suppression resulting in neutropenia observed. Interestingly, 11 patients with HC before the clinical trial with CDV were treated with supportive care only, and all demonstrated complete resolution of HC. The persistence of BK viruria during CDV therapy and after resolution of HC manifestations, although with a decreased viral load, calls into question the adequacy of CDV therapy for BKV–related HC, because the most clinically appropriate measurement of the efficacy of antiviral treatment is clearance of virus and lack of progression to disease. Indeed, in the HSCT setting, clinical resolution of adenovirus or CMV infection following CDV treatment is associated with sustained clearance of these viruses [39,40]. We do not know whether decreased BK viral load translates into clinical improvement, however. The presumed therapeutic effect in some patients, which might be biased by a concomitant immune recovery, together with the lack of known treatments and the in vitro activity of CDV against BK virus, suggest the need for further evaluation of CDV therapy for BK virus–related HC.

In the present study, despite a higher rate of reactivation of BKV in the urine in our HSCT recipients, approximately one-third of the patients developed BKV–related late-onset HC. These findings confirm our hypothesis that higher urinary BK viral load is not a sole risk factor for late-onset HC development, and that other cofactors, such as ATG use and grade II-IV aGVHD, contribute significantly to this complication. Multicenter prospective randomized trials are needed to further define the role of CDV therapy in BKV–related HC.

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