The incidence of hemorrhagic cystitis and BK-viruria in allogeneic hematopoietic stem cell recipients according to intensity of the conditioning regimen

Géraldine Giraud
Gordana Bogdanovic
Peter Priftakis
Mats Remberger
Britt-Marie Svahn
Lisbeth Barkholt
Olle Ringden
Jacek Winiarski
Per Ljungman
Tina Dalianis

The influence of BK-viruria, donor background, and conditioning on the development of hemorrhagic cystitis was examined in 90 allogeneic hematopoetic stem cell transplant patients, of whom 15 developed hemorrhagic cystitis. Thirty-two patients had related and 58 had unrelated donors, while 44 received full, and 46 received reduced intensity conditioning (RIC). BK-viruria was more common in patients with hemorrhagic cystitis than in those without ($p<0.01$), and hemorrhagic cystitis was less common in patients with related donors than in those with unrelated donors ($p=0.02$). Finally, hemorrhagic cystitis and BK-viruria were less common in patients receiving RIC, rather than full conditioning ($p<0.01$ and $p<0.01$, respectively).

Key words: BK virus, hemhorragic cystitis, predictive factors.

Haematologica 2006; 91:401-404

From the Department of Oncology-Pathology, Karolinska Institutet (GG, GB, PP, TD), Department of Clinical Microbiology (GB), Department of Paediatrics (PP, JW), Centre for Allogeneic Stem Cell Transplantation (B-MS, LB, OR), Haematology Centre (PL), Department of Clinical Immunology (MR, TD), Karolinska University Hospital, Stockholm, Sweden.

Correspondence:
Tina Dalianis, Department of Oncology-Pathology, Karolinska Institutet, CCK, R8:01, Karolinska University Hospital, 171 76 Stockholm, Sweden.
E-mail: tina.dalianis@cck.ki.se

Reactivation of BK virus (BKV) infection[1,2] has been associated with late onset (>2 weeks post-transplantation) hemorrhagic cystitis in allogeneic hematopoietic stem cell transplant (HSCT) recipients.[3-5] Hemorrhagic cystitis occurs as microscopic hematuria (grade I), or as gross hematuria with clots and urinary tract obstruction (grades II–IV) and causes significant morbidity and mortality.[6] From 5-40% of all HSCT recipients develop hemorrhagic cystitis, while 50-100% of the patients have BK-viruria indicating that BK-viruria alone is not sufficient for inducing hemorrhagic cystitis.[6-9] Acute graft-versus-host disease (GVHD) and specific BKV mutations have been suggested to influence the development of hemorrhagic cystitis, and patients with hemorrhagic cystitis often excrete a higher load of BKV in their urine than do patients without hemorrhagic cystitis. [7-13] Moreover, patients with hemorrhagic cystitis frequently exhibit BK-viruria, accompanied by a high viral load together with acute GVHD, prior to or at the onset of the hemorrhagic cystitis.[14] It has also been reported recently that recipients of graft from unrelated donors have a higher risk of hemorrhagic cystitis than have recipients of grafts from related donors.[15] Whether conditioning prior to HSCT affects the incidence of BKV-associated hemorrhagic cystitis has not been studied so far, although adenovirus-associated hemorrhagic cystitis was demonstrated to be milder and of shorter duration in patients receiving reduced intensity conditioning (RIC).[16] Hence in this study we investigated the influence of full myeloblative conditioning or RIC, prior to HSCT, on the development of BKV-associated hemorrhagic cystitis. We also assessed other risk factors for hemorrhagic cystitis such as donor source, BK-viruria, viral load, and GVHD.

Design and Methods

Patients

Ninety allogeneic HSCT recipients transplanted between April 2002 and July 2004 at the Karolinska University Hospital, of whom 31 were described previously,[14] were included in this study, which was conducted with ethical approval 357/01 from the Karolinska Institutet. Fifteen patients developed late onset hemorrhagic cystitis grade II-IV.[6] Forty-four (49%) of the patients received RIC regimens; fludarabine, (90-150 mg/m^2) in combination with 6 Gy fractionated total body irradiation (f-TBI) and 60 mg/kg cyclophosphamide, or in combination with 2 Gy f-TBI, 8 mg/kg busulphan, or 60 mg/kg cyclophosphamide or treosulphan, or 20 mg/kg cyclophosphamide (for patients with Fanconi's anemia). The other 46 (51%) received full myeloablative conditioning; 12Gy f-TBI and cyclophosphamide, busulphan and cyclophosphamide. For the purpose of this study, patients with aplastic anemia receiving cyclophosphamide 120 mg/kg were defined as having received a myeloablative regimen (Table 1). Fifty-eight patients (64%) received a transplant from an unrelated donor, while 32 (36%) received a graft from a related donor. Details of the patients' characteristics, donor background, conditioning regimen and grade of GVHD,[17] as correlated with the development of hemorrhagic cystitis are presented in Table 2.

BKV detection and quantification in urine samples

Three hundred urine samples (1-28 samples/patient) were collected from the second week onwards after HSCT at weekly intervals during hospitalization and thereafter once a month. Samples were tested (2.5 and 5 μL), for BKV DNA by nested polymerase chain reaction (detection ≥10 BKV copies).[18,19] BKV positive samples, diluted (1:1, 1:10, 1:50, 1:100, 1:500) to avoid possible inhibition, were quantified for viral load in triplicate by quantitative real time polymerase chain reaction analysis. [20]

Statistical analysis

Statistical significance was calculated by the χ^2 test and multivariate logistic regression with forward selection of variables. In the multivariate model, only samples for BKV detection collected before or simultaneously with the development of hemorrhagic cystitis were taken into account. The numbers of urine samples were compared with the Mann-Whitney U-test.

Results and Discussion

Fifteen patients with hematologic diseases developed late onset hemorrhagic cystitis (Table 2), 16-95 days after HSCT. Most of these patients, (10/15; 67%) developed the hemorrhagic cystitis in the first month after HCST. The hemorrhagic cystitis was severe (grades 3-4) in 12/15 patients (80%), while it was moderate to severe (grade 2) in 3/15 patients (20%). Thirteen of the 15 patients (87%) had received a full myeloablative conditioning regimen indicating that RIC is associated with a decreased risk for hemorrhagic cystitis (p<0.01) (Table 2). This difference remained statistically significant (p=0.04) even if the 16 patients with solid tumours were excluded from the analysis.

Fifty-eight patients (64%) were transplanted with a graft from an unrelated donor, while 32 patients (36%) received a graft from a related donor. Of the 58 recipients of unrelated grafts, 29 (50%) were conditioned with myeloablative conditioning, while 29 (50%) received RIC. Fourteen of the unrelated graft recipients developed hemorrhagic cystitis, while only one patient transplanted with a graft from a related donor did so, (p=0.02), indicating that hemorrhagic cystitis is more common in recipients of grafts from unrelated donors.

BK-viruria was more common in patients with hemorrhagic cystitis (13/15; 87%) than in those without (26/75; 35%) (p<0.01). The difference was independent of the fact that more samples were analyzed for patients with hemorrhagic cystitis (94 total, median 3 samples/patient) than for those without (206 total, median 2 samples/patient) (p=0.03). BK-viruria was observed in 11/13 patients (85%) before the onset of hemorrhagic cystitis, in 9/9 patients (100%) during hemorrhagic cystitis, and in 10/10 patients (100%) after the cystitis indicating that the sampling time point was not a factor responsible for the significant difference between patients with and without hemorrhagic cystitis. In addition, BK-viruria was more frequent in patients receiving full conditioning 25/44 (57%) than in patients receiving RIC 14/46 (30%) (p<0.01), while it was similar in patients transplanted with grafts from unrelated donors (24/58; 41%) or a related donors (15/32; 47%) (p=NS). Notably, there were no differences in the number of urine samples between patients conditioned with a RIC or a myeloablative regimen or between patients transplanted from an unrelated or a related donor (p=NS). A viral load >10^6 BKV copies/μL urine was more common in patients with hemorrhagic cystitis (9/15; 60%) than without (13/75; 17%) (p<0.01). There was no difference in the risk for having a viral load >10^6 BKV copies/μL urine for patients receiving full conditioning (14/47; 30%) compared to patients receiving RIC (8/43; 19%) (p=NS) or in patients transplanted from an unrelated donor (14/58; 24%) or a related donor (8/32; 25%) (p=NS).

Acute GVHD was observed in 60/90 (67%) of patients (Table 2) and 24 (28%) patients developed grade II-IV acute GVHD (*data not shown*). There was no difference in the frequency of acute GVHD in patients with hemorrhagic cystitis (12/15; 80%) and without (48/75; 64%) (p=NS). The corresponding numbers for grade II-IV acute GVHD were 6/15 (40%) and 18/75 (24%), respectively (p=NS). There was also no differ-

Table 1. Clinical characteristics and donor background of 90 HSCT recipients, evaluated for hemorrhagic cystitis in relation to their conditioning regimen.

	Myeloablative RIC	*RIC*
Patients	46	44
Sex (Male/Female)	30/16	23/21
Median age (Min-Max)	52 (6-64)	13 (0.7-59)°
Donors		
Related	17	15
Unrelated	29	29
Underlying Diagnosis		
Leukemia	18	36
Lymphoproliferative disorder	7	1
Non-malignant disorder	3	5
Others	2	2
Solid tumor	16	0
Disease stage		
Early disease/late disease	10/20	21/23
GVHD prophylaxis		
CsA+MTX	39	34
Other combinations	7	10
Conditioning		
Flu+fTBI 6Gy +Cy	7	
Flu+TBI 2 Gy	1	
Flu+Bu	17	
Flu+Cy	19	
Flu+treosul	2	
fTBI 12Gy+Cy		18
Bu+Cy		24
Cy		2
Anti-thymocyte globulin	39	32
Stem cell source (BM/PBSC/CB)	9/36/1	19/22/3
$CD34^+$ dose (10^6/kg)	7.8 (0.6-28.0)	7.2 (0.1-66.0)

**p<0.05, °p<0.01; early disease; First complete remission, first chronic phase and non-malignant disease/late disease; all others, solid tumors not included. CsA; cyclosporine, MTX; methotrexate, Flu; fludarabine, fTBI; fractionated total-body irradiation, Cy; cyclophosphamide, Bu; busulphan, ATG; BM; bone marrow, PBSC; peripheral blood stem-cells; CB; cord blood.*

Table 2. Clinical characteristics, donor background and conditioning regimen of 90 HSCT recipients evaluated for hemorrhagic cystitis and in relation to hemorrhagic cystitis development.

	hemorrhagic cystitis	*non HC (BK pos)*
Patients	15	75(26)
Sex (Male/Female)	11/4	45/30 (16/10)
Age		
0-18 y	6	21 (10)
>18 y	9	54 (16)
Underlying disease		
Leukemia	13	41 (17)
Lymphoprolif. disorder	8 (2)	
Non-malignant disorder	1	7 (3)
Others	1	3
Solid tumor	16 (4)	
Donor		
Related	1	31 (11)
Unrelated	14	44 (11)
Conditioning regimen		
Full myeloablative	13	31(17)
Reduced intensity	2	44 (11)
AcuteGVHD		
Grade 0	3	27 (8)
Grade I-IV	12	48 (18)

BKV-pos, number of patients with a BKV positive urine sample at some time during the study period who did not develop hemorrhagic cystitis. Underlying disease, leukemia: (acute lymphoblastic leukemia, n=15; acute myeloid leukemia, n=18; chronic myeloid leukemia, n=9; chronic lymphocytic leukemia, n=1; acute myeloid leukemia secondary to myelodysplastic syndrome, n=4; myelodysplastic syndrome, n=7), lymphoproliferative disorders (myeloma, n=1; lymphoma, n=1; non-Hodgkin's lymphoma, n=6), non-malignant disease (severe aplastic anemia, n=5; Fanconi's anemia, n=3), others (PNH, n=1), polycythemia vera, n=1; juvenile myelomonocytic leukemia, n=1; AMN/ALD, n=1), solid tumors (liver, n=5; kidney, n=4; prostate, n=2; colon, n=2; rectum, n=3).

ence in the frequency of acute GVHD between patients who received full myeloablative conditioning (32/44; 73%) or RIC (28/46; 61%) (p=NS). Furthermore, there was no difference in the incidence of acute GVHD between patients transplanted from an unrelated donor (38/58; 66%) or a related donor (22/32; 69%) (p=NS). In multivariate logistic regression analysis, BK-viruria before the onset of hemorrhagic cystitis (OR 7.4; 1.7-33.2; p<0.01) and a graft from an unrelated donor (OR 20.0; 2.0-198; p=0.01) increased the risk for hemorrhagic cystitis while RIC conditioning reduced the risk (OR; 0.06; 0.01-0.42; p<0.01). A BK-viral load >10^6 copies/μL urine was not an independent factor associated with hemorrhagic cystitis when BK-viruria was included in the model. As mentioned above, late onset hemorrhagic cystitis is an important complication of allogeneic HSCT, and previous studies have evaluated risk factors for late onset hemorrhagic cystitis in addition to BK-viruria.[6,8,10-12]

In this study, the intensity of the conditioning regimen, the donor source and the presence of BK-viruria were all identified as predictive factors for the development of hemorrhagic cystitis. Of the 15 patients, who did develop hemorrhagic cystitis all but two had received a full conditioning regimen, indicating that RIC decreases the risk of hemorrhagic cystitis; a finding that has not been previously reported. The pathogenesis of late onset hemorrhagic cystitis after HSCT is still poorly understood. We have previously shown that allogeneic HSCT carries a higher risk of hemorrhagic cystitis than does autologous HSCT.[10] It seems likely that the immunosuppressed status of the patient is also important since patients transplanted with grafts from unrelated donors have a higher risk of hemorrhagic cystitis than do recipients of grafts from related donors.[15] Our finding that RIC HSCT gives a lower risk than myeloablative HSCT sheds further light on the issue. One possible explanation is that the combination of a harsher regimen together with the presence of BKV increases the risk of hemorrhagic cystitis. Another possibility, taking into account the donor type is that the more immunosuppressive regimen given to recipients of grafts from unrelated donor together with a high intensity regimen and BK-viruria combine to create the environment in which hemorrhagic cystitis develops. Finally, a greater initial preservation of the recipient's immunity and thereafter a more gradual switch to complete donor chimerism may facilitate a gradual adaptation of the immune response to BKV. This is supported by our finding that BK-viruria was significantly less common in patients receiving RIC and, as reported earlier, BK-viruria and viral load >10^6 BKV copies/μL urine were more common in patients with hemorrhagic cystitis than in those without.[7,8,13] However, the latter suggestion must be cautiously interpreted, since a statistically significant difference in BKV load was not noted between patients undergoig RIC or full conditioning, although a lower proportion of RIC patients had a high BKV load in their urine samples.

In summary, our results show that patients who receive a transplant from an unrelated donor and have full myeloblative conditioning have an increased risk of hemorrhagic cystitis. We conclude that the use of RIC may minimize the incidence of hemorrhagic cystitis.

GG, GB, PP, MR, BS, LB, OR, JW: substantial contributions to the conception and design of the study, or acquisition analysis and interpretation of data; GB, PL, TD: drafting the article or revising it critically for important intellectual content; TD: final approval of the version to be submitted. The authors declare they have no potential conflict of interest.

The Children's Cancer Foundation, the Swedish Cancer Foundation, the Stockholm Cancer Society, Karolinska Institutet, and The Stockholm City Council are acknowledged for financial support. The authors thank Parviz Kokhaei for valuable technical advice, Raja Choudhury, for critically reviewing the manuscript, and the patients and the staff at the Centre of Allogeneic Stem Cell Transplantation and the Departments of Haematology and Paediatrics.

Manuscript received September 28, 2005. Accepted December 27, 2005.

References

1. Chesters PM, Heritage J, McCance DJ. Persistence of DNA sequences of BK virus and JC virus in normal human tissues and in diseased tissues. J Infect Dis 1983;147:676-84.
2. Reploeg MD, Storch GA, Clifford DB. BK virus: a clinical review. Clin Infect Dis 2001;33:191-202.
3. Apperley JF, Rice SJ, Bishop JA, Chia YC, Krausz T, Gardner SD, et al. Late-onset hemorrhagic cystitis associated with urinary excretion of polyomaviruses after bone marrow transplantation. Transplantation 1987; 43: 108-12.
4. Arthur RR, Shah KV, Baust SJ, Santos GW, Saral R. Association of BK viruria with hemorrhagic cystitis in recipients of bone marrow transplants. N Engl J Med 1986;315:230-4.
5. Azzi A, Fanci R, Bosi A, Ciappi S, Zakrzewska K, de Santis R, et al. Monitoring of polyomavirus BK viruria in bone marrow transplantation patients by DNA hybridization assay and by polymerase chain reaction: an approach to assess the relationship between BK viruria and hemorrhagic cystitis. Bone Marrow Transplant 1994; 14:235-40.
6. Bedi A, Miller CB, Hanson JL, Goodman S, Ambinder F, Charache P, et al. Association of BK virus with failure of prophylaxis against hemorrhagic cystitis following bone marrow transplantation. J Clin Oncol 1995;13:1103-9.
7. Azzi A, Cesaro S, Laszlo D, Zakrzewska K, Ciappi S, de Santis R, et al. Human polyomavirus BK (BKV) load and haemorrhagic cystitis in bone marrow transplantation patients. J Clin Virol 1999;14:79-86.
8. Leung AY, Suen CK, Lie AK, Liang RH, Yuen KY, Kwong YL. Quantification of polyoma BK viruria in hemorrhagic cystitis complicating bone marrow transplantation. Blood 2001;98:1971-8.
9. Seber A, Shu XO, Defor T, Sencer S, Ramsay N. Risk factors for severe hemorrhagic cystitis following BMT. Bone Marrow Transplant 1999;23:35-40.
10. Bogdanovic G, Ljungman P, Wang F, Dalianis T. Presence of human polyomavirus DNA in the peripheral circulation of bone marrow transplant patients with and without hemorrhagic cystitis. Bone Marrow Transplant 1996;17:573-6.
11. Bogdanovic G, Priftakis P, Taemmeraes B, Gustafsson A, Flaegstad T, Winiarski J, et al. Primary BK virus (BKV) infection due to possible BKV transmission during bone marrow transplantation is not the major cause of hemorrhagic cystitis in transplanted children. Pediatr Transplant 1998; 2: 288-93.
12. Ost L, Lonnqvist B, Eriksson L, Ljungman P, Ringden O. Hemorrhagic cystitis: a manifestation of graft versus host disease? Bone Marrow Transplant 1987;2:19-25.
13. Biel SS, Held TK, Landt O, Niedrig M, Gelderblom HR, Siegert W, et al. Rapid quantification and differentiation of human polyomavirus DNA in undiluted urine from patients after bone marrow transplantation. J Clin Microbiol 2000;38:3689-95.
14. Bogdanovic G, Priftakis P, Giraud G, Kuzniar M, Ferraldeschi R, Kokhaei P, et al. Association between a high BK virus load in urine samples of patients with graft-versus-host disease and development of hemorrhagic cystitis after hematopoietic stem cell transplantation. J Clin Microbiol 2004; 42: 5394-6.
15. El-Zimaity M, Saliba R, Chan K, Shahjahan M, Carrasco A, Khorshid O, et al. Hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation: donor type matters. Blood 2004;103:12:4674-80.
16. Yamamoto R, Kusumi E, Kami M, Yuji K, Hamoki T, Saito A et al. Late hemorrhagic cystitis after reduced-intensity hematopoietic stem cell transplantation (RIST) Bone Marrow Transplant 2003;32:1089-95
17. Glucksberg H, Storb R, Fefer A Buchner CD, Nieman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA matched sibling donors. Transplantation 1974;295-304.
18. Bogdanovic G, Brytting M, Cinque P, Grandien M, Fridell E, Ljungman P, et al. Nested PCR for detection of BK virus and JC virus DNA. Clin Diagn Virol 1994;2:211-20.
19. Hammarin AL, Bogdanovic G, Svedhem V, Pirskanen R, Morfeldt L, Grandien M. Analysis of PCR as a tool for detection of JC virus DNA in cerebrospinal fluid for diagnosis of progressive multifocal leukoencephalopathy. J Clin Microbiol 1996;34:2929-32.
20. Priftakis P, Bogdanovic G, Kokhaei P, Mellstedt H, Dalianis T. BK virus (BKV) quantification in urine samples of bone marrow transplanted patients is helpful for diagnosis of hemorrhagic cystitis, although wide individual variations exist. J Clin Virol 2003;26:71-7.