was observed (liver and spleen) and in organs rich in blood perfusion (heart, kidney and peripheral blood). These differences might contribute to the observed tissue specificities, although this is speculative because the data are only from one marmoset. Bone marrow was negative for BCR-ABL. We believe that our inability to detect BCR-ABL expression in this sample was because we could obtain insufficient hematopoietic cells from the fibrotic bone marrow. Recently, a mutation in JAK2 gene was reported to cause myelofibrosis [33]. We hypothesized that the lentiviral vector inserted into the host genome and unregulated the expression of genes such as JAK2. We performed LAM-PCR (linear amplification-mediated PCR), which identifies the sequence flanking the integrated vector genome, to identify the unregulated gene that may be responsible for myelofibrosis [34]. However, due to the insufficient bone marrow samples, this attempt was unsuccessful. If the phenomenon is reproduced in other marmosets, further analyses will be required to understand this pathology.

In conclusion, we stably expressed an oncogene *in vivo* in a marmoset model, although several steps may be required to develop hematological malignancy in this model. The results provide information that can be used to establish a marmoset disease model in which hematopoietic stem/progenitor cells are targeted for oncogene delivery.

5. Acknowledgements

We thank Dr. Hajime Ishii (Central Institute for Experimental Animals, Kawasaki, Japan) for advice on common marmosets, Dr. Hiroyuki Miyoshi (Bioresource Center, RIKEN, Tsukuba, Japan) for providing the lentiviral vector system and Dr. Yoshikazu Sugimoto (Keio University, Tokyo, Japan) for performing LAM-PCR. We thank Mr. Keisuke Takahashi, Ms. Sanae Suzuki and members of our laboratory for assistance. We thank Dr. Kazuaki Yokoyama and Ms. Nozomi Yusa (The University of Tokyo) for technical assistance with the p190 BCR-ABL quantitative RT-PCR. We thank Dr. Nobukazu Watanabe (The University of Tokyo) for technical assistance with flow cytometry. Finally, we thank the Iwaki Scholarship foundation for helping with the overseas studies of Y.D.

This study was supported by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare of Japan.

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Leukemia & Lymphoma, July 2012; 53(7): 1423–1424

ISSN: 1042-8194 print / 1029-2403 online
DOI: 10.3109/10428194.2011.653787

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LETTER TO THE EDITOR

Helicobacter pylori-induced thrombocytosis clinically indistinguishable from essential thrombocythemia

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Eradication of *Helicobacter pylori* is becoming a first-line therapy in patients with idiopathic thrombocytopenic purpura (ITP). The relationship between the immune destruction of platelets and *H. pylori* infection has been extensively discussed, but still remains unclear. On the other hand, a high frequency of gastrointestinal lesions and higher susceptibility to *H. pylori* have been reported in patients with myeloproliferative neoplasms (MPNs) including essential thrombocythemia (ET) [1,2], while the clinical outcome of patients with ET after eradication of *H. pylori* is totally unknown. Here we report, with bibliographic consideration, a 66-year-old female with putative ET, which was dramatically ameliorated by *H. pylori* eradication therapy alone.

A 66-year-old female patient with Hashimoto disease and Sjögren syndrome had been undergoing levothyroxine replacement therapy for over 25 years. At the age of 60, her peripheral platelet count had increased to $87.5 \times 10^4/\mu L$. A bone marrow study showed normocellular marrow with an increased number of megakaryocytes, but either JAK2 V617F or c-MPL W515L mutation tested negative. Soon after this, her peripheral platelet count increased to over $100 \times 10^4/\mu L$. She was suffering from autoimmune disorders which could have caused chronic inflammation, but the level of her platelet count could not be explained by reactive thrombocytosis to inflammation. Further diagnostic procedures confirmed that there was no evidence of a tumor possibly producing thrombopoietin and interleukin-6, and so on. She was tentatively diagnosed as having ET and began to receive 1000 mg/day hydroxycarbamide (HU) and 100 mg/day acetylsalicylic acid for anti-platelet therapy. The platelet count was maintained at around $80 \times 10^4/\mu L$ (Figure 1). At the age of 65, she developed an intractable skin ulcer at the left external malleolus which was suspected to be an adverse effect of HU, and hence intravenous injection of ranimustine (MCNU) was substituted for HU, but only once.

Just after the ranimustine admnistration, she was referred to our hospital and then continued on only acetylsalicylic acid. During this period she complained of dyspepsia, and for the first time underwent a gastrointestinal endoscopic

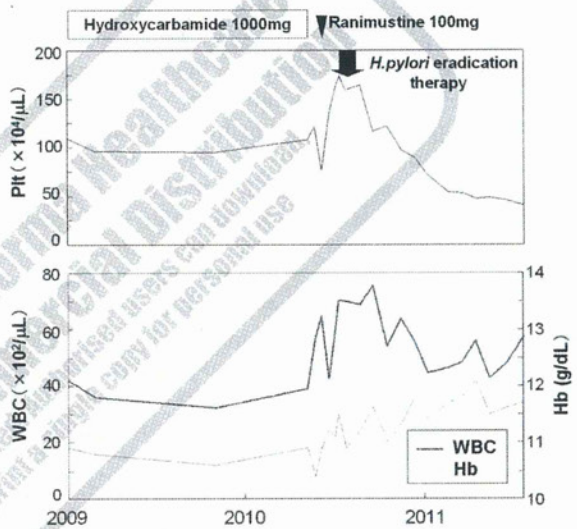

Figure 1. Changes in platelet count before and after *H. pylori* eradication therapy. The platelet count increased after discontinuation of hydroxycarbamide (HU), but has been gradually decreasing to the normal range after *H. pylori* eradication therapy in September 2010.

study, which indicated that she had chronic active gastritis. Since she was highly positive for anti-*H. pylori* immunoglobulin G (IgG), 1-week eradication therapy consisting of lansoprazole (60 mg/day), clarithromycin (400 mg/day) and amoxicillin (1500 mg/day) was performed, and eradication of *H. pylori* was confirmed by the rapid urease test. The platelet count at the start of therapy was $164 \times 10^4/\mu L$, and 4 weeks later it had decreased to $115.6 \times 10^4/\mu L$. Thereafter, the platelet count continued to decrease gradually, and 1 year after the eradication therapy the platelet count was constantly below $40 \times 10^4/\mu L$, almost within the normal range, without any chemotherapeutic drugs. The present clinical course strongly suggests reactive thrombocytosis induced by *H. pylori* infection, instead of ET.

ITP results from the accelerated destruction of peripheral platelets by a platelet-specific autoimmune reaction and

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Received 10 November 2011; revised 22 December 2011; accepted 24 December 2011

additional impairment of platelet production. The efficacy of *H. pylori* eradication therapy in patients with ITP was first reported in 1998 [3], and it is now becoming a first-line therapy. With regard to the pathogenetic relationship between *H. pylori* infection and ITP development, several explanations have been proposed according to the following clinical data: (1) a subset of anti-platelet autoantibodies, which might cross-react with the *H. pylori*-derived cytotoxin-associated gene A (CagA) protein, disappeared after eradication therapy [4]; (2) monoclonal antibodies against *H. pylori* urease B cross-reacted with the platelet glycoprotein IIIa [5]; and (3) monocytes from *H. pylori*-infected patients showed enhanced phagocytic activity and reduced expression of the inhibitory Fcγ receptor IIB (FcγRIIB) [6]. Nevertheless, the true pathogenesis remains to be elucidated.

There are paradoxical reports that infection with *H. pylori* increases the number of peripheral platelets in patients without ITP, which can be reversed by eradication therapy [7,8]. This observation is supported by experimental data that *H. pylori*-induced chronic inflammation can up-regulate the synthesis of interleukin-6 by local macrophages [9], which in turn stimulates platelet production directly or indirectly through up-regulation of thrombopoietin [10]. However, platelet counts are generally much less than $100 \times 10^4/\mu L$ in reactive thrombocytosis, except for cases bearing certain tumors that constitutively produce megakaryocyte-stimulating factors such as thrombopoietin and interleukin-6, and our patient is not such a case. The revised World Health Organization criteria for diagnosis of ET includes the demonstration of JAK2 V617F or other clonal marker, or no evidence of reactive thrombocytosis in the absence of JAK2 V617F. Recent advances in genomic analysis of MPN have identified many mutations in additional genes including CBL, LNK, SOCS, TET2, ASXL1, EZH2 and IDH, which may contribute to the pathogenesis of MPN and offer novel clonal markers [11]. Although evidence of clonality was not demonstrated in this case, the initial diagnosis of ET was probable according to the sustained platelet count over $100 \times 10^4/\mu L$ in the absence of documented inflammation or tumor. In this case, the titer of serum anti-*H. pylori* IgG was extremely high (199 U/mL; upper limit 9.9 U/mL) at the start of therapy, then decreased over time, but still remains moderate (34 U/mL) 1 year after therapy. Thus, one possible explanation for the pathogenesis of *H. pylori*-induced thrombocytosis is that a subset of anti-*H. pylori* IgG might work as a thrombopoietin-mimetic molecule to stimulate its downstream signal.

In conclusion, to our knowledge, this is the first reported case of *H. pylori*-induced thrombocytosis closely resembling ET. Now, we plan to conduct a clinical study of screening for *H. pylori* infection in patients with apparent thrombocytosis that lacks defined molecular abnormalities to confirm a correlation between *H. pylori* infection and thrombocytosis. It is worthwhile trying *H. pylori* eradication therapy in patients with putative ET who have no clonal markers such as JAK2 V617F mutation and have definite signs for *H. pylori* infection.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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Leukemia Research 36 (2012) 128–131

Contents lists available at SciVerse ScienceDirect

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Unrelated cord blood transplantation after myeloablative conditioning regimen in adolescent and young adult patients with hematologic malignancies: A single institute analysis

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ARTICLE INFO

Article history:
Received 21 July 2011
Received in revised form 5 September 2011
Accepted 17 September 2011
Available online 5 October 2011

Keywords:
Cord blood transplantation
Adolescence
Hematopoietic malignancy
Myeloablative conditioning

ABSTRACT

We report the results of unrelated cord blood transplantation (CBT) after myeloablative conditioning regimen in 16 patients with hematologic malignancies from 15 to 20 years old. The median times of myeloid and platelet engraftment were 21 and 38 days, respectively. The cumulative incidences of acute graft-*vs*-host disease (GVHD) was 62.0%, all of which were grade I or II, and that of extensive-type chronic GVHD was 12.5%. The probabilities of overall and disease-free survival at 3 years were 68.2% and 48.6%, respectively, comparable to adult or childhood cases. Adolescents and young adult patients with hematologic malignancies who have no HLA-matched adult donors could be considered as candidates for CBT.

1. Introduction

Recently the medical needs of adolescents and young adults with hematologic malignancies have become better defined. In comparison of outcome of patients with 16–21 years of age with acute lymphoblastic leukemia (ALL) treated with pediatric *vs* adult clinical trials, pediatric trials yielded better outcome than adult trials [1]. In patients with acute myelocytic leukemia (AML), outcome of children younger than age 15 years has significantly improved for the last several decades, but that of patients with 15–19 years remains poor [2]. Thus, adolescents and young adults with hematologic malignancies are distinct in terms of their therapeutic requirements compared to adults or children. However, there have been no data defined adolescent and young adult patients for cord blood transplantation (CBT) after conventional myeloablative conditioning regimen. We here first report the clinical results for a group of 16 adolescent and young adult patients with hematologic malignancies treated with CBT in our institute, showing the safety and efficacy comparable to those for adults and children.

2. Patients and methods

This is a retrospective single-center analysis. Between September 1999 and July 2009, 16 patients at adolescent and young adult ages from 15 to 20 years old were treated with CBT as the first allogeneic stem cell transplantation at The Research Hospital, Institute of Medical Science, University of Tokyo. One patient received an autologous bone marrow transplantation before he had come to our hospital. Written informed consent for treatment was obtained from all patients with the Declaration of Helsinki. Patients were qualified as being standard risk and high risk according to the criteria in the previous reports [3,4].

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doi:10.1016/j.leukres.2011.09.016

2.1. HLA typing and donor selection

HLA-A and HLA-B antigens were identified by serological typing. HLA-DRB1 alleles were determined by high-resolution molecular typing using polymerase chain reaction sequence-specific primers. Patients who did not have HLA-matched family or unrelated adult donors were considered to be eligible for CBT. In the selection of cord blood unit for transplantation, all cord blood grafts were evaluated by HLA-A, HLA-B and HLA-DRB1 typing, and nucleated cell counts. Preferred cord blood units matched 4 of 6 to 6 of 6 HLA loci and contained a minimal cell count of 1.5×10^7 nucleated cells/kg body weight before freezing according to the criteria of our institution as shown in the previous reports [3,4]. All cord blood units were obtained from cord blood banks belonging to the Japan Cord Blood Bank Network.

2.2. Conditioning regimen, GVHD prophylaxis, and supportive care

All patients received fractionated 12 Gy total body irradiation and chemotherapy as a myeloablative conditioning regimen (Table 1). Fifteen patients received standard cyclosporin (CsA) and methotrexate (MTX), and one patient received CsA alone for a graft-*vs*-host disease (GVHD) prophylaxis [3,4]. Fifteen mg/m^2 of MTX was given intravenously on day 1, and 10 mg/m^2 on days 3 and 6 as previously reported [3,4]. Both acute and chronic GVHD (aGVHD and cGVHD, respectively) were graded according to the previously published criteria [5,6]. The criterion to stop immunosuppression depended on patients' disease status. All patients received recombinant human granulocyte colony-stimulating factor starting on day 1 until durable granulocyte recovery was achieved.

2.3. Endpoints and statistical analysis

The chimerism status after CBT, engraftment, graft failure, treatment-related mortality (TRM), and disease-free survival (DFS) were defined as described in the previous reports [3,4].

Data analysis was performed on 1 December 2010. The probability of overall survival (OS) and DFS were estimated using the Kaplan–Meier method.

3. Results and discussion

The characteristics of the 16 patients and the cord blood units are shown in Table 1. Six patients were classified as standard risk while 10 patients as high risk. Six patients (2 ALL, 3 myelodysplastic syndrome (MDS) and 1 chronic myelocytic leukemia (CML)) were initially treated by Pediatric units. All patients received a single and HLA-mismatched cord blood unit. The median numbers of cryopreserved nucleated cells and CD34$^+$ cells were 2.50×10^7/kg (range 2.05 to 3.73×10^7/kg) and 0.94×10^5/kg (range 0.46 to 1.33×10^5/kg), respectively. The median numbers of infused nucleated and CD34$^+$ cells were 2.11×10^7/kg ($n = 11$; range 1.36 to 2.38×10^7/kg) and 0.76×10^5/kg ($n = 11$; range 0.25 to 2.55×10^5/kg), respectively.

Fourteen patients (87.5%) successfully achieved myeloid reconstitution and 2 patients went into graft failure regardless of above of median number of total nucleated cells (2.71 and 2.45×10^7/kg, respectively) and CD34$^+$ cells (1.09 and 1.13×10^5/kg, respectively) transplanted. One had full recovery with 100% of host chimerism by day 52, and the other took a second cord blood graft on day 30. All patients with myeloid reconstitution showed full donor chimerism at the first bone marrow examination after CBT. The median time to an absolute neutrophil count $>0.5 \times 10^9$/L among the patients with engraftment was 21 days (range 19–32 days). The cumulative

Table 1
Characteristics of patients, cord blood units, and outcomes.

Characteristics	
Patients, *n*	16
Male/female, *n*	9/7
Median age, years (range)	17 (15–20)
Median weight (kg) (range)	52 (45–71)
Median number of cryopreserved nucleated cells $\times 10^7$/kg (range)	2.50 (2.05–3.73)
Median number of cryopreserved CD34$^+$ cells $\times 10^5$/kg (range)	0.94 (0.46–1.33)
Median number of infused nucleated cells, *n* $\times 10^7$/kg (range)	11 2.11 (1.36–2.38)
Median number of infused CD34$^+$ cells, *n* $\times 10^5$/kg (range)	11 0.76 (0.25–2.55)
Median time from diagnosis to transplantation Days (range)	429 (65–1898)
Recipient CMV status, positive/negative, *n*	13/3
Diagnosis	
De novo AML [*n* (%)]	3 (19)
CR1, *n*	1
CR2, *n*	1
Not in remission, *n*	1
ALL [*n* (%)]	7 (44)
CR1, *n*	2
CR2, *n*	4
CR3, *n*	1
CML BC [*n* (%)]	1 (6)
MDS [*n* (%)]	4 (25)
RA, *n*	1
RCMD, *n*	1
Advanced (*n*)	2
MDS/MPD [*n* (%)]	1 (6)
Disease status at transplant*	
Standard risk [*n* (%)]	6 (37)
High risk [*n* (%)]	10 (63)
Conditioning regimen	
TBI + CY + AraC, *n*	1
TBI + CY + AraC/G-CSF, *n*	9
TBI + CY, *n*	4
TBI + CY + Tepa, *n*	1
TBI + Flu + Mel, *n*	1
GVHD prophylaxis	
CSP + sMTX, *n*	15
CSP, *n*	1
Number of HLA-A, B, DRB1 mismatches	
1, *n*	4
2, *n*	5
3, *n*	6
4, *n*	1
Engraftment [day (range)]	
Median time to neutrophil count $>0.5 \times 10^9$/L	21 (19–32)
Median time to platelet count $>50 \times 10^9$/L	38 (33–98)
Acute GVHD [*n* (%)]	
0	1 (7)
Grade I	6 (43)
Grade II	7 (50)
Grade III	0
Grade IV	0
Chronic GVHD [*n* (%)]	
None	0
Limited	10 (83)
Extensive	2 (17)
Immunosuppressant termination ($n = 7$)	
Median time [day (range)]	267 (83–952)
Cause of death [*n* (%)]	
Relapse	4 (80)
MOF	1 (20)

CMV, cytomegalovirus; AML, acute myelogenous leukemia; CR1, CR2, CR3: first, second, third complete remission, respectively; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; BC, blast crisis; MDS, myelodysplastic syndrome; RA, refractory anemia; RCMD, refractory cytopenia with multilineage dysplasia; Advanced, patients with MDS-related secondary AML; MPD, myeloproliferative disease; TBI, total body irradiation; Ara-C, cytosine arabinoside; G-CSF, granulocyte colony-stimulating factor; CY, cyclophosphamide; Tepa, thiotepa; Flu, fludarabine; Mel, melphalan; CsA, cyclosporine; sMTX, short-term methotrexate; MOF, multiple organ failure.

* Patients qualified as being standard risk or high risk according to the criteria described in previous reports [3,4].

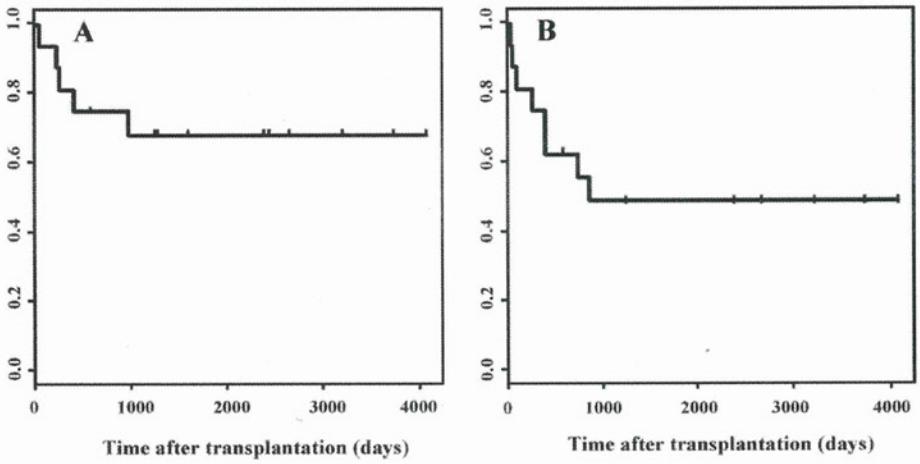

Fig. 1. Kaplan–Meier estimates of overall survival (A) and disease-free survival (B) after cord blood transplantation in adolescent patients with hematologic malignancies. The probability of OS (A) and DFS (B) at 3 years was 67.5% (95% CI, 47.6–95.8%) and 48.6% (95% CI, 29.0–81.4%), respectively.

incidence of neutrophil recovery at day 42 was 87.5% (95% confidence interval (CI), 68.9–100%). A self-sustained platelet count $>50 \times 10^9$/L was achieved in 13 patients at a median time of 38 days (range 33–98 days). The cumulative incidence of platelet recovery at day 100 was 81.3% (95% CI, 57.3–100%). These results using single units showed that the hematopoietic reconstituting ability of unrelated CB in adolescent patients was similar to those in pediatric and adult ones [7–10].

aGVHD occurred in 13 of 14 evaluable patients who survived for more than 100 days, but there were no patients with grades III and IV aGVHD (Table 1). The cumulative incidence of grade I or II aGVHD at day 100 was 62.0% (95% CI, 27.8–96.2%). cGVHD occurred in all of 12 evaluable patients, and 2 among them displayed the extensive type. The cumulative incidence of cGVHD and extensive-type cGVHD at 1 year was 68.8% (95% CI, 43.6–94.0%) and 12.5% (95% CI, 0–29.4%), respectively. There was no relationship between the immunosuppressive therapy and the occurrence of aGVHD and cGVHD. TRM only occurred in one patient. This patient suddenly suffered an infarction in the pons on day 22 after CBT, and died of multiple organ failure (MOF) on day 43. The cumulative incidence of TRM at 1 year was very low (6.3%). Consequently GVHD was not related to TRM in our study. The relatively higher incidence of GVHD in our study may come from earlier discontinuation of immunosuppressant as reported previously [3,4].

However, 2 of 6 standard risk patients and 5 of 10 high risk patients relapsed. The cumulative incidence of relapse at 3 years was 45.1% (95% CI, 18.8–71.4%). All of the relapsed patients received chemotherapy to obtain remission, and five patients received a second CBT. To find the risk factors to relapse after CBT in adolescent and young adult patients, we analyzed the relationships between the relapse and the numbers of infused nucleated and $CD34^+$ cells, GVHD prophylaxis and the number of HLA-mismatches, but there were no significant relationships. In the patients with ALL, 1 out of 2 patients, who were treated by pediatric-type regimen before CBT, and 3 out of 5 patients, who were treated by non-pediatric-type regimen, relapsed. Although it was shown that pediatric-type regimen is favorable for the treatment of adolescent and young adult patients in ALL [1], there was no significant relationship between the relapse after CBT and the treatment with pediatric-type regimen in this study. However, since the number of patients was small in our single institute analysis, further study with a larger number of patients may be needed to find the risk factor to relapse after CBT.

Eight patients are alive and disease-free at the median 97 months (range 20–135 months) after transplantation. The disease-free patients included the patient who underwent autologous bone marrow recovery without relapse. All of the alive patients had a good performance status with 90–100% Karnofsky score at the time of analysis. The probabilities of OS and DFS at 3 years were 67.5% (95% CI, 47.6–95.8%) and 48.6% (95% CI, 29.0–81.4%), respectively (Fig. 1A and B). The probability of DFS at 3 years in the standard risk patients was 66.7% (95% CI, 37.9–100%) while that in the high risk group was 40% (95% CI, 18.7–85.5%). Atsuta et al. reported that OS and DFS at 2 years in adult cases who received CBT were 48% and 42% in AML, and 52% and 46% in ALL, respectively [11]. Eapen et al. also showed that DFS at 2 years was 44% in remission cases and 15% in non-remission cases at CBT in adults [12]. Pediatric studies reported that OS at 2 years was 45.5%[9] and DFS at 5 years was 33–60% [10]. Thus, both DFS and OS in the present study were comparable to those in adult and pediatric patients.

However, in the previous report by our institute, the 3-year probability of DFS after unrelated CBT for hematological malignancies was higher (70%) than that in the present report, especially in the standard risk patients (93% *vs* 67%) [4]. In comparison with the previous report, the overall rate of high risk patients (62% *vs* 57%), the 1-year incidence of TRM (6% *vs* 9%), the 100-day incidence of aGVHD (62% *vs* 52%) and the cumulative incidence of cGVHD in patients surviving more than 100 days (69% *vs* 71%) were almost similar. In contrast, the 3-year cumulative incidence of relapse was significantly higher in the present study (45% *vs* 17%). Therefore, the difference in DFS between the present and previous reports might have been caused by the biological characteristics of hematological malignancies in adolescence, such as the resistance of malignant cells to anti-cancer drugs or immunological immaturity reducing the graft-*vs*-malignant cell effect. Accordingly, further improvement in the pre-transplantation chemotherapy, conditioning regimen and post-transplantation immunomodulation may be needed to achieve better outcomes during the treatment of adolescent hematologic malignancies with unrelated CBT.

In summary, although our patient cohort was small, our results suggested that CBT after myeloablative conditioning regimen could be safe for adolescents and young adult patients with hematologic malignancies as well as pediatric and adult patients. However, since the adolescent hematologic malignancies are thought to be relatively chemoresistant, a therapeutic regimen that takes the biological characteristics of these malignancies into account would contribute to achieve better outcomes.

Conflicts of interest statement

Authors have no conflict of interest to disclose to the current manuscript.

Acknowledgments

The authors would like to thank the pediatricians, physicians, and nurses who cared for patients, Ms. Maki Monna for data management. This work was supported in part of The Kobayashi Foundation.

Contributions. Y.E., S.T., A.T., S.A., and K.T. designed the study; Y.E., S.M., S.K., T.K., and J.O. performed patients' care; K.Y. and F.N. analyzed data statistically; and Y.E. and K.T. wrote the paper.

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TRANSPLANT
INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

A case–control study of bronchiolitis obliterans syndrome following allogeneic hematopoietic stem cell transplantation

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Keywords

ABO-mismatch, allogeneic hematopoietic stem cell transplantation, bronchiolitis obliterans syndrome, cord blood, graft-versus-host disease.

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Conflicts of interest
The authors report no potential competing conflicts of interest.

Received: 31 October 2012
Revision requested: 5 January 2013
Accepted: 23 February 2013

doi:10.1111/tri.12093

Summary

Bronchiolitis obliterans syndrome (BOS) is a significant complication after allogeneic hematopoietic stem cell transplantation (HSCT). However, the pathogenesis and risks for the development of BOS have remained unclear. Therefore, a case–control study was conducted to investigate the risk factors for the development of BOS, which included the largest number of BOS cases; 196 patients with BOS were identified and compared with 1960 control recipients. The following were identified as significantly higher risk factors for the development of BOS: female recipients (OR 1.47, $P = 0.019$), ABO-mismatch HSCT (minor mismatch, OR 1.67, $P = 0.015$; major mismatch, OR 1.73, $P = 0.012$; bidirectional mismatch, OR 1.96, $P = 0.018$), busulfan+cyclophosphamide-based myeloablative conditioning (OR 1.74, $P = 0.016$), and acute graft-versus-host disease (GVHD) involving the skin (OR 1.55, $P = 0.011$). On the other hand, the risk for the development of BOS was significantly lower in patients receiving cord blood transplantation (OR 0.26, $P = 0.0011$). With respect to other target organs of chronic GVHD, ocular involvement was significantly associated with BOS (OR 2.53, $P < 0.001$). Prospective studies are required to elucidate the risk factors for the development of BOS, and future investigations should focus on finding a prophylactic approach against BOS based on these findings.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) plays a crucial role as a curative treatment for hematological diseases. However, HSCT recipients experience various adverse complications, including graft-versus-host disease (GVHD). Bronchiolitis obliterans syndrome (BOS) is one of the significant late complications following HSCT, and it is known to represent lung involvement of chronic GVHD (cGVHD). BOS is characterized by breathing difficulty and dry cough without fever, and by airway obstruction not responsive to bronchodilator therapy that may become irreversible in advanced stages of disease [1–7]. The pathological findings of BOS show bronchiolitis involving the small airway and fibrinous obliteration of the lumina of the respiratory bronchioles [3,8]. The cumulative incidence of BOS is thought to range from 2% to 10% [3,4]. BOS usually presents after the first 100 days following HSCT, and ~80% of cases present between 6 and 12 months after HSCT [3,4]. The International Bone Marrow Transplantation Registry (IBMTR) reported that BOS presented at a median of 431 days after HSCT (range: 65–2444 days) [9].

Several groups have investigated the risk factors for the development of BOS, including peripheral blood stem cell transplantation (PBSCT), busulfan (BU)-based conditioning, and the development of GVHD [9–13]. However, the results were controversial. One of the reasons for the controversy is the small number of patients with BOS, as almost all of these studies included less than 20 patients with BOS. To the best of our knowledge, there have been just two reports that included more than 50 patients with BOS by IBMTR (76 patients with BOS among 6275 HSCT recipients from HLA-identical siblings) or the Kanto Study Group for Cell Therapy (KSGCT, 57 patients with BOS among 2087 recipients). However, no study has included over 100 patients with BOS [9,13]. Both IBMTR and KSGCT reported that PBSCT and GVHD were associated with the development of BOS. However, it remains unclear whether other alternative donor sources, such as cord blood transplantation (CBT), and other possible factors, such as ABO-mismatch, affect the development of BOS.

Bronchiolitis obliterans syndrome is well known to impair the recipients' quality of life dramatically and to be associated with worse survival rates [1,3,4,6,13]. However, an effective treatment has yet to be established [1,3,4,6,13]. Therefore, it is important to elucidate the risks for the development of BOS and to establish a prophylactic approach against it. Thus, a large case–control study that included about 200 patients with BOS was performed using the Japanese transplant outcome registry database, and the risk factors were identified.

Patients and methods

Patient selection

Patients with BOS and control recipients were selected from the cohort of adult recipients (16 years or older) who received their 1st allogeneic HSCT between January 1990 and December 2009 and survived without disease relapse for at least 180 days after HSCT, reported to the Japan transplant outcome registry database and confirmed by the Transplant Registry Unified Management Program in 2010 [14]. The BOS patients were defined as adult recipients who experienced BOS by their last follow-up. The control recipients were defined as adult recipients in whom BOS was not apparently diagnosed up to their last follow-up. Using a computerized selection procedure, 10 controls, which were matched according to years of HSCT (every 5 years), were chosen for each case, because there might be changes in the clinical practices related to HSCT according to the years of HSCT. In addition, information on age, sex, and survival status at the end of follow-up was required. This retrospective analysis was conducted in accordance with the amended Declaration of Helsinki and approved by the institutional review board at Saitama Medical Centre, Jichi Medical University.

Definitions of categories

BOS was reported based on clinical obstructive dysfunctions and radiological assessment with/without histological examinations [2,5,7]. Standard risk diseases were defined as follows: acute leukemia in the 1st and 2nd complete remission, chronic myelogenous leukemia in the 1st and 2nd chronic phase, lymphoma and multiple myeloma in complete and partial remission, adult T cell leukemia in complete remission, myelodysplastic syndromes, myeloproliferative neoplasms, benign hematological diseases, and congenital disorders. All other diseases were classified as high-risk. Because PBSCT from unrelated donors was not available in Japan during the evaluation period, the types of HSCT were categorized into seven groups: HLA-matched related bone marrow transplantation (MRD-BMT), HLA-mismatched related BMT (MMRD-BMT), HLA-matched related PBSCT (MRD-PBSCT), HLA-mismatched related PBSCT (MMRD-PBSCT), HLA-matched unrelated BMT (MUD-BMT), HLA-mismatched unrelated BMT (MMUD-BMT), and unrelated CBT. MMRD or MMUD was defined as a related or unrelated donor when at least HLA 1 antigen mismatch was detected at serological levels of HLA-A, B, or DR. Regimens were classified into myeloablative (MAC) and reduced intensity conditioning (RIC) based on the report by Giralt *et al.* [15]. Briefly, conditionings including total body irradiation (TBI) >8 Gy, melphalan $\geq$ 140 mg/m^2, or oral BU $\geq$ 9 mg/kg (iv BU $\geq$ 7.2 mg/kg) were classified

as MAC. Other regimens were classified as RIC. The conditioning regimens were then divided into five groups: cyclophosphamide (CY)+TBI-based MAC, BU+CY-based MAC, other MAC, fludarabine-based RIC, and other RIC. The diagnosis and severity of GVHD were reported based on the clinical grading scores [16,17].

Statistical analysis

Conditional logistic regression analysis was used for univariate and multivariate analyses to assess the risks for the development of BOS. On multivariate analysis, odds ratios (ORs) were obtained after adjusting with variables having a *P*-value less than 0.1 on univariate analysis with stepwise deletions. Acute GVHD (aGVHD) was included in the analysis as a possible risk factor for the development of BOS, because BOS usually presents after the first 100 days after HSCT [3,4]. In addition, the association between BOS and the target organs of cGVHD was assessed separately by focusing on the recipients with cGVHD. The cumulative probabilities of relapse and nonrelapse mortality (NRM) were estimated by Gray's method, considering each other as a competing risk. Overall survival (OS) was estimated by the Kaplan–Meier method. These probabilities were estimated from time of transplantation with 95% confidence intervals (95% CIs). Statistical significance was defined as a two-tailed *P*-value less than 0.05. All data management and statistical calculations were performed by STATA version 12.0 and EZR on R commander, which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0) (Saitama Medical Centre, Jichi Medical University at http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html).

Results

Patients

During the 20-year study period, 196 patients with BOS (96 males, 100 females) were identified. The median age of the BOS group was 41 (range 16–68) years. Through the computerized selection procedure described above, 1960 control patients (1149 males, 841 females) were identified among 6595 eligible recipients who survived for at least 180 days after HSCT. Their median age was 40 (range 16–76) years. There was no significant difference in the distributions of age and disease risk between the BOS and control groups.

Risk factors for the development of BOS

On univariate analyses, the risk for the development of BOS was higher in female recipients, ABO-mismatch HSCT (especially major mismatch), recipients receiving BU+CY-based MAC, those who experienced grade 2–4, and skin involvement of aGVHD. On the other hand, the risk for the development of BOS was lower in the recipients who received unrelated CBT and *in vivo* T cell depletion, including anti-thymocyte globulin and alemtuzumab, as part of conditioning (Table 1). HLA mismatch, sex-mismatch, GVHD prophylaxis, and gut and liver involvement of aGVHD were not associated with the development of BOS in the current analysis.

Multivariate analysis revealed that the predictive factors for the development of BOS were as follows: female recipients [OR 1.47 (95% CI; 1.06–2.04), $P = 0.019$], ABO-mismatch [minor mismatch, OR 1.67 (95% CI; 1.10–2.51), $P = 0.015$; major mismatch, OR 1.73 (95% CI; 1.13–2.64), $P = 0.012$; bidirectional mismatch, OR 1.96 (95% CI; 1.12–3.43), $P = 0.018$], CBT [OR 0.26 (95% CI; 0.11–0.58), $P = 0.0011$], BU+CY-based MAC [OR 1.74 (95% CI; 1.11–2.72), $P = 0.016$], and skin involvement of aGVHD [OR = 1.55 (95% CI; 1.11–2.18), $P = 0.011$] (Table 1). Grade 2–4 aGVHD and *in vivo* T cell depletion were not significant on multivariate analysis.

The association between BOS and target organs of cGVHD

For the 1118 recipients who experienced cGVHD, the information on the other target organs of cGVHD was available in 113 patients in the BOS group and 834 control recipients. The 113 patients accounted for 4% of the eligible prematched patients with cGVHD ($n = 2743$). BOS was associated with ocular involvement [OR = 2.53 (95% CI; 1.62–3.95), $P < 0.001$] and oral involvement [OR = 1.52 (95% CI; 1.00–2.33), $P = 0.051$]. On multivariate analysis, only ocular involvement was significant (Table 2). Naturally, the BOS group included more extensive cGVHD (88% vs. 63%, $P < 0.01$).

Relapse, nonrelapse mortality, and survival of patients with BOS

The median follow-up duration of the survivors with BOS was 1538 (range 200–6048) days. Of the 196 recipients with BOS, 107 died during the study period. The estimated 4-year OS in the BOS group was 51% (95% CI 43–58%) (Fig. 1). Of the 107 deaths, the proportion of relapse death was 8.8% (15 of 107). Of the remaining 92 nonrelapse deaths, fatal respiratory failure as a result of BOS accounted for 53% (49 of 92) of the causes of death in the BOS group. Other fatal pulmonary events were observed in 4% (4 of 92): acute respiratory distress syndrome in 3% (3 of the 92 nonrelapse deaths) and interstitial pneumonia in 1% (1 of 92). Other nonpulmonary causes of nonrelapse death were infection in 20% (18 of 92), cGVHD other than pulmonary

Table 1. Impact of patient and transplant characteristics on bronchiolitis obliterans syndrome.

	BOS		Control		Univariate		Multivariate	
	N	%	*N*	%	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Total	196	100	1960	100				
Sex								
Male	96	49	1119	57	1		1	
Female	100	51	841	43	1.38 (1.03–1.85)	0.031	1.47 (1.06–2.04)	0.019
Age (years)								
16–49	141	72	1378	70	1		NA	
50 and more	55	28	582	30	0.92 (0.65–1.29)	0.62	NA	
Disease								
Leukemia	165	84	1604	82	1		–	
Lymphoma	21	11	188	10	1.09 (0.68–1.77)	0.72	–	
Plasma cell neoplasm	2	1	35	2	0.56 (0.13–2.34)	0.43	–	
Marrow failure	3	2	108	6	0.27 (0.08–0.86)	0.026	–	
Others	5	3	25	1	1.92 (0.73–5.07)	0.19	–	
Disease risk								
Standard	149	76	1474	75	1		NA	
High	43	22	477	24	0.89 (0.63–1.27)	0.53	NA	
Missing	4	2	9	0				
CMV sero-status								
Negative	26	13	297	15	1		NA	
Positive	133	68	1356	69	1.14 (0.73–1.77)	0.56	NA	
Missing	37	19	307	16				
Sex match								
Match	86	44	1008	51	1		NA	
Male to female	49	25	417	21	1.34 (0.93–1.94)	0.12	NA	
Female to male	41	21	441	23	1.10 (0.74–1.63)	0.64	NA	
Missing	20	10	94	5				
ABO-mismatch								
Match	80	41	1013	52	1		1	
Minor mismatch	40	20	386	20	1.29 (0.87–1.92)	0.21	1.67 (1.10–2.51)	0.015
Major mismatch	39	20	339	17	1.46 (0.97–2.18)	0.069	1.73 (1.13–2.64)	0.012
Bidirectional mismatch	19	10	171	9	1.37 (0.80–2.33)	0.25	1.96 (1.12–3.43)	0.018
Missing	18	9	51	3				
Types of transplant								
MRD-BMT	43	22	445	23	1		1	
MMRD-BMT	7	4	78	4	0.89 (0.38–2.06)	0.78	0.64 (0.24–1.72)	0.38
MRD-PBSCT	40	20	318	16	1.21 (0.74–1.98)	0.44	1.28 (0.76–2.16)	0.35
MMRD-PBSCT	10	5	77	4	1.31 (0.62–2.81)	0.48	1.45 (0.65–3.22)	0.36
MUD-BMT	69	35	612	31	1.09 (0.71–1.68)	0.68	1.09 (0.69–1.72)	0.71
MMUD-BMT	6	3	85	4	0.69 (0.28–1.72)	0.42	0.58 (0.23–1.49)	0.26
CBT	8	4	307	16	0.26 (0.12–0.57)	<0.001	0.26 (0.11–0.58)	0.0011
Missing	13	7	38	2				
Conditioning								
CYTBI	83	42	843	43	1		1	
BUCY	43	22	274	14	1.68 (1.12–2.52)	0.011	1.74 (1.11–2.72)	0.016
Other MAC	26	13	219	11	1.25 (0.78–1.99)	0.36	1.40 (0.84–2.32)	0.19
Flu-based RIC	35	18	481	25	0.72 (0.48–1.09)	0.12	0.73 (0.47–1.14)	0.17
Other RIC	9	5	135	7	0.68 (0.34–1.39)	0.29	0.68 (0.31–1.46)	0.32
Missing	0	0	8	0				
In vivo T cell depletion								
None	193	98	1845	94	1		–	
Presence	3	2	115	6	0.25 (0.079–0.80)	0.019	–	
GVHD prophylaxis								
CsA-based	123	63	1167	60	1		NA	
Tac-based	67	34	751	38	0.83 (0.60–1.15)	0.25	NA	

Table 1. continued

	BOS		Control		Univariate		Multivariate	
	N	%	*N*	%	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Others	5	3	41	2	1.20 (0.46–3.12)	0.72	NA	
Missing	1	1	1	0				
Grade of acute GVHD								
0–1	107	55	1243	63	1		–	
2–4	88	45	714	36	1.44 (1.07–1.94)	0.017	–	
Missing	1	1	3	0				
Target of acute GVHD								
Skin								
No	73	37	867	44	1		1	
Present	122	62	1056	54	1.38 (1.02–1.87)	0.04	1.55 (1.11–2.18)	0.011
Missing	1	1	37	2				
Gut								
No	145	74	1502	77	1		NA	
Present	47	24	411	21	1.19 (0.84–1.69)	0.32		
Missing	4	2	47	2				
Liver								
No	183	93	1787	91	0.99 (0.54–1.83)	0.98	NA	
Present	12	6	120	6				
Missing	1	1	53	3				

BOS, bronchiolitis obliterans syndrome; CI, confidence interval; CMV, cytomegalovirus; MRD, HLA-matched related donor; MMRD, HLA-mismatched related donor; MUD, HLA-matched unrelated donor; MMUD, HLA-mismatched unrelated donor; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation; CBT, cord blood transplantation; CY, cyclophosphamide; TBI, total body irradiation; BU, busulfan; MAC, myeloablative conditioning; Flu, fludarabine; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporine; Tac, tacrolimus; NA, not assessed. "Marrow failure" includes aplastic anemia, pure red cell aplasia, and paroxysmal nocturnal hemoglobinuria. The "Other diseases" group includes EB virus-associated diseases, solid tumor, hemophagocytic syndrome, primary immunodeficiency, congenital metabolic disorders, and others.

involvement in 8% (7 of 92), organ failure other than respiratory failure in 7% (6 of 92), thrombotic microangiopathy in 1% (1 of 92), hemorrhage in 1% (1 of 92), and other unknown causes in 7% (6 of 92). The estimated 4-year NRM in the BOS group was 38% (95% CI 30–45%) (Fig. 2).

Discussion

A case–control study that included the largest number of recipients with BOS reported so far was performed, and the risk factors for the development of BOS were identified retrospectively. The risk for the development of BOS was significantly higher in female recipients, ABO-mismatch HSCT, recipients receiving BU+CY-based MAC, and those who experienced aGVHD involving the skin. On the other hand, the risk was significantly lower in patients receiving CBT. As the factors included in the analysis were pretransplant or supposed as events before the onset of BOS, the association was thought to be predictive factors.

To the best of our knowledge, this analysis is the first to reveal the adverse impact of ABO-mismatch on the development of BOS in the HSCT setting. It is well known that ABO-mismatch is critically associated with graft rejection in solid organ transplants [18,19]. Not only major but also minor ABO-mismatch organ transplant is supposed to have an increased risk for graft rejection, severe hemolysis, and lower survival rates, although it is controversial [18–26]. Similarly, both of the major and minor ABO-mismatches in HSCT were also reported to have an adverse impact on the incidence of GVHD and NRM [27]. BOS following HSCT is one manifestation of lung cGVHD and resembles chronic graft rejection after lung transplant. Taking all of these into consideration, it is plausible that ABO-mismatch has a potential to induce lung injuries in the HSCT setting [3,5]. The possible mechanism might be a direct capture on lung epithelial cells of anti-recipient-A/B antibodies produced by donor B cells in the minor ABO-mismatch HSCT setting [28,29]. Another possible mechanism might be through inflammation and activation of adhesion molecules induced by the destruction of donor-derived red blood cells and complexes with the allo-/auto-reactive antibodies produced by recipient remnant B cells in the major ABO-mismatch HSCT setting [30–32]. These inflammatory conditions are well observed in intravascular hemolysis, resulting in thrombosis and platelet activation [33,34]. Recently, rituximab has been reported to be a promising strategy in ABO-mismatch organ transplant to prevent graft rejection [35]. Therefore, rituximab might also affect the development of BOS in the ABO-mismatch HSCT setting.

Table 2. The association between bronchiolitis obliterans syndrome and target organs of chronic GVHD.

	BOS *N*	Control *N*	Univariate Odds ratio (95% CI)	*P*-value	Multivariate Odds ratio (95% CI)
Target organs of cGVHD	113	834			
Eye					
None	62	603	1	<0.0001	2.53 (1.62–3.95)
Present	51	231	2.53 (1.62–3.95)		
Mouth					
None	50	463	1	0.051	-
Present	63	371	1.52 (1.00–2.33)		
Skin					
None	35	309	1	0.21	NA
Present	78	525	1.32 (0.85–2.06)		
Liver					
None	66	463	1	0.83	NA
Present	47	371	0.96 (0.62–1.46)		
Mucosa/gut					
None	82	659	1	0.25	NA
Present	38	204	1.33 (0.82–2.15)		
Joint/muscle					
None	105	798	1	0.13	NA
Present	8	36	1.67 (0.67–4.18)		
Hair					
None	110	811	1	0.7	NA
Present	3	23	0.78 (0.23–2.71)		
Serositis					
None	111	820	1	0.75	NA
Present	2	14	0.78 (0.17–3.56)		
Other involvement					
None	107	789	1	0.54	NA
Present	6	45	0.75 (0.29–1.89)		

BOS, bronchiolitis obliterans syndrome; cGVHD, chronic graft-versus-host disease; NA, not assessed; "Other involvement" includes nephropathy, neuropathy, weight loss, thrombocytopenia, and other involvement.

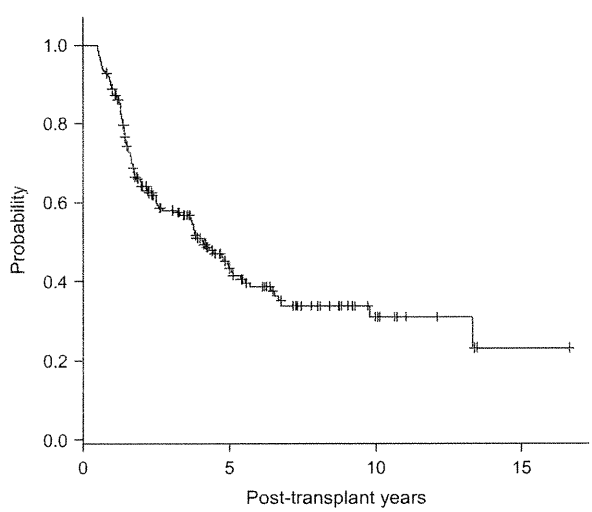

Figure 1 Overall survival of recipients with bronchiolitis obliterans syndrome from time of transplant.

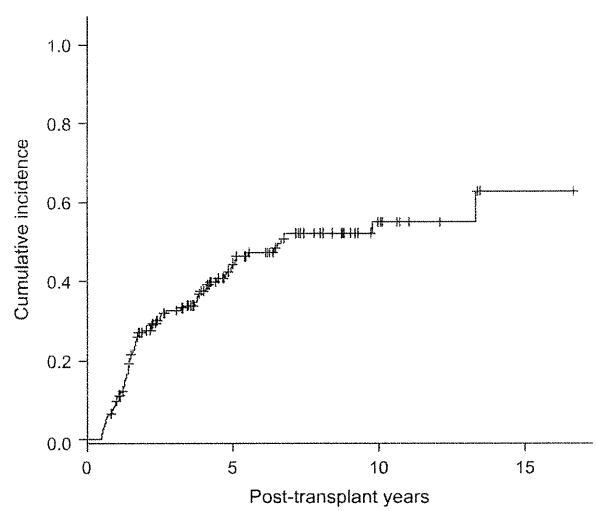

Figure 2 Nonrelapse mortality of recipients with bronchiolitis obliterans syndrome from time of transplant.

Lung injury as a result of conditioning toxicity is also one of the proposed mechanisms for the development of BOS [9,10,12,36]. Of the various conditioning regimens, BU-CY-based MAC was identified as a significant risk factor for the development of BOS in this study, which was consistent with the results of previous reports [9,10,36]. High concentrations of BU might contribute to lung injuries and the development of BOS, as well as liver injuries, inducing veno-occlusive disease [37].

Another possible mechanism for the development of BOS is probably caused by allo-reactive immune responses. Allo-reactive donor T cells might target lung epithelial cells, inducing BOS as one of the manifestations of cGVHD in the lungs. In fact, GVHD and the possible risk factors for GVHD have been reported to be associated with the development of BOS in several studies [4,9,13,36]. In this study, it was also found that recipients who experienced grade 2–4 aGVHD and skin involvement of aGVHD had a significantly higher risk for the development of BOS on univariate analyses, although grade 2–4 aGVHD was not significant on multivariate analysis. The close relation between skin and lung complication might exist in HSCT setting as well as in connective tissue disease [38]. In addition, the development of BOS was associated with ocular involvement of cGVHD when focusing on recipients with cGVHD. However, it should be noted that the association between BOS and each target organ of cGVHD was assessed separately, and it was not known whether the ocular involvement of cGHVD developed earlier than BOS. This 20-year database included many recipients before NIH consensus 2005 [7]. Therefore, specific-organ involvements might be under diagnosed.

This is the first study to suggest that CBT was significantly associated with a lower risk for the development of BOS, although there was no association between PBSCT and the development of BOS. It is known that the incidences of acute and cGVHD in the CBT group are significantly lower than in the unrelated BMT group [39]. Therefore, the low incidence of GVHD might be attributable to the low incidence of BOS in the CBT group. A prospective study is needed to verify the favorable impact of CBT on the development of BOS. On the other hand, HLA mismatch and sex-mismatch, which are also reported as important risk factors for acute and cGVHD, had little impact on the development of BOS in the current analysis.

This analysis had several limitations as a result of its retrospective nature, and all information was based on the reports by attending physicians, not on a central review. First, the severity of BOS could not be assessed because the data of pulmonary function test were not available from the registry data. Second, it was not possible to assess the time-dependent impact of BOS on relapse and survival rates because the dates of BOS development were also not available. Third, because the study period was so long that the details mentioned above could not be fully collected although we realize the importance. Truly, only prospective cohort studies adhering to strict diagnostic criteria and other clinical data will be able to shed the light into the factors associated with the incidence and outcomes of BOS. However, the strength of this study is that it involved the largest number of recipients with BOS of all studies to date. Therefore, the detailed impact of conditioning regimens, stem cell sources, and ABO-mismatches could be analyzed. In addition, we obtained similar results even when we re-analyzed the risk factors for the development of BOS among the eligible entire cohort or a selected cohort between 2005 and 2009 for which few information were missing (data not shown).

In summary, the risk factors for the development of BOS included: female recipients, ABO-mismatch transplantation, BU+CY-based MAC, and skin involvement of aGVHD. On the other hand, the risk of BOS was significantly lower in recipients receiving CBT. Prospective studies are required to elucidate the risk factors for the development of BOS, and future investigations should focus on the development of a prophylactic approach against BOS based on these findings.

Authorship

HN: designed the study, analyzed data, and wrote the manuscript. JK, SY, YA and TM: advised on methods, analyzed data, and wrote the manuscript. HA, TF, KK, TA, TY, ST and JT: collected data. YM, TN and HS: collected data and were responsible for the data management of JMDP, JCBBN and JSHCT, respectively. MM: analyzed data, wrote the manuscript, and was responsible for the study and GVHD-WG of the JSHCT.

Funding

This study was supported in part by a Health and Labour Science Research Grant (Research on Allergic Disease and Immunology) from the Ministry of Health, Labour and Welfare of Japan (M.M.).

Acknowledgements

The authors would like to express their appreciation for work of all of the physicians and data managers at the centers that contributed valuable data on transplantation to the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP), and the Japan Cord Blood Bank Network (JCBBN). They would also like to thank all of the members of the Transplant Registry Unified Management committees in the JSHCT, the

JMDP, and the JCBBN for their dedicated data management.

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Impact of hepatitis C virus infection on clinical outcome in recipients after allogeneic hematopoietic cell transplantation

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The impact of hepatitis C virus (HCV) infection on outcomes following allogeneic hematopoietic cell transplantation (HCT) remains a matter of debate. We have retrospectively examined the significance of HCV infection among recipients who received allogeneic HCT, using a Japan transplant outcome registry database between 2006 and 2009. Among 7,831 recipients, 136 were HCV-positive. The rate of hematopoietic recovery was lower in the HCV-positive group (neutrophil recovery of 500 × 10^9/L or higher: 79% vs. 87% at Day 30, $P = 0.087$; platelet recovery of 50 × 10^9/L or higher: 57% vs. 65% at Day 60, $P = 0.012$). The HCV-positive group had a significantly higher incidence of nonrelapse mortality 38% vs. 25% at 2 years, $P < 0.01$) and inferior overall survival (41% vs. 51% at 2 years, $P < 0.01$). A multivariate analysis revealed that HCV seropositivity was associated with an independent risk for higher nonrelapse mortality (hazard ratio: 1.65, $P < 0.01$) and inferior overall survival (hazard ratio: 1.39, $P < 0.01$). The incidences of death due to hepatic problems (8% vs. 2%, $P < 0.01$), bacterial infection (10% vs. 4%, $P < 0.01$), or graft failure (5% vs. 2%, $P = 0.084$) tended to be higher in the HCV-positive group. HCV infection had an adverse impact on the clinical outcome following HCT, especially in the setting of unrelated transplantation. Careful evaluation before embarking on HCT and intensive assessment against complications are warranted in HCV-infected recipients. Am. J. Hematol. 00:000–000, 2013. © 2013 Wiley Periodicals, Inc.

Introduction

Since allogeneic hematopoietic cell transplantation (HCT) was introduced about 50 years ago, the procedure has spread widely because of its potential to cure hematological diseases [1]. Recent progress in HCT has been associated with the development of stem cell sources such as peripheral blood or cord blood, alternative donors, novel strategies of immunosuppression, and reduced-intensity conditioning (RIC) regimens. However, many recipients often experience various complications, including organ failure, infection, and acute and chronic graft-versus-host disease (aGVHD and cGVHD, respectively). The identification of risk factors for these complications may help to further improve transplant outcomes.

The hepatitis C virus (HCV) was identified in 1989 [2]. It is estimated that over 2 million and 130–170 million people suffer from HCV infection in Japan and worldwide, respectively [3–5]. Because of HCV infection, chronic hepatitis, liver cirrhosis, or hepatocellular carcinoma can develop during long-term follow-up [6]. Previously, HCV was transmitted mainly by blood exposure, such as by transfusion, but recent systematic screening has reduced the proportion of transfusion-transmitted infection [7,8]. However, HCV has remained an important clinical concern because HCV-positive recipients represent 6% of long-term survivors even in the postscreening era [7,8].

The impact of HCV on HCT outcomes has remained a matter of debate. Early retrospective studies in 1990s showed that HCV infection was not associated with an increased risk for either long-term mortality or liver complications among bone marrow transplant (BMT) survivors for at least 2 years [9,10], and these results were verified by a prospective 10-year observation that included both allogeneic and autologous HCT [11]. Therefore, HCV infection was not considered a major problem in HCT for a long time [12]. However, a long-term observation revealed that HCT recipients with HCV progressed to cirrhosis more rapidly than non-HCT patients with HCV [7,13]. Furthermore, a recent case–control study by a Brazilian group reported that HCV infection was an independent risk factor for inferior survival [14]. This discrepancy may be due to the small numbers of HCV recipients and the differences in patient backgrounds. Most of these studies included less than 50 HCV-positive recipients before HCT. In addition, most of the earlier studies in the 1990s included younger recipients (a median of less than 30 years) and few, if any, cases of HCT other than related BMT [9–11,13,15]. On the other hand, the patients in the recent study by the Brazilian group included relatively older recipients (a median of 49

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Conflicts of interest: Nothing to report.

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Received for publication 26 February 2013; Accepted 5 March 2013

Am. J. Hematol. 00:000–000, 2013.

Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.23436

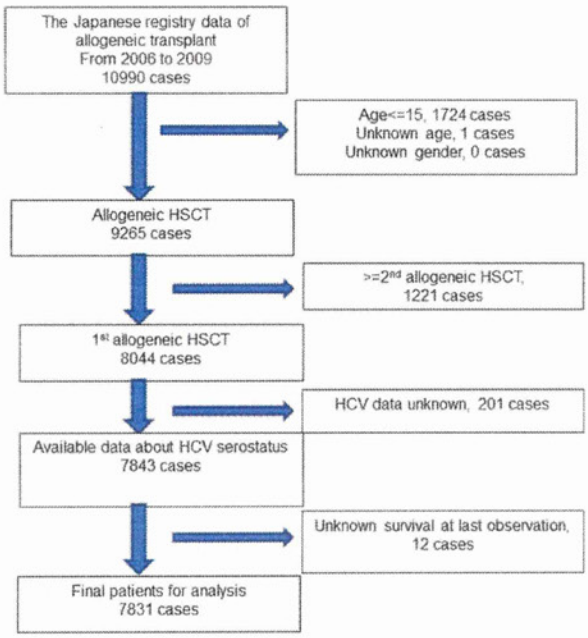

Figure 1. Scheme of patient selection. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

years) and more patients who received transplant from unrelated donors (10 of 31 HCV-positive recipients) [14].

As recent progress in the modalities of HCT has spread the indications for allogeneic HCT, the impact of HCV on the clinical outcome needs to be reassessed among recent HCT patients according to the patient background.

Patients and Methods

Patient selection

The patient data were obtained from the Japan transplant outcome registry database by the Transplant Registry Unified Management Program confirmed in 2010 [16]. Eligible patients included all adult recipients (16 years or older) who received their first allogeneic HCT between January 2006 and December 2009 and for whom information on age, gender, HCV serostatus at transplantation, and survival status at last observation were available (Fig. 1). The median duration of follow-up for survivors was 691 days after HCT. This retrospective analysis was conducted in accordance with the amended Declaration of Helsinki and approved by the institutional review board at Saitama Medical Centre, Jichi Medical University.

Definitions of categories

HCV infection was reported according to the presence of anti-HCV antibody. European group for blood and marrow transplant (EBMT) risk score was recalculated as far as we could according to the previous report [17]. Since peripheral blood stem cell transplantation (PBSCT) from an unrelated donor was not available in Japan in the era of this study, HCT was categorized into three groups: related BMT/PBSCT, unrelated BMT, and unrelated cord blood transplantation (CBT). HLA mismatch was defined as incompatibility between the recipient and donor when at least a one-antigen mismatch was detected at serological levels of HLA-A, B, or DR. The intensity of conditioning was classified as myeloablative conditioning (MAC) or RIC determined based on the report by Giralt et al. [18]. Briefly, regimens that included TBI >8 Gy, melphalan ≥ 140 mg/m^2, or oral busulfan ≥ 9 mg/kg (iv busulfan ≥ 7.2 mg/kg) were classified as MAC. Other regimens were classified as RIC [18]. Neutrophil recovery was defined as the continuous achievement of neutrophil counts of 500×10^6/L or higher. Platelet recovery was also assessed from the perspective of the achievement of platelet counts of 50×10^9/L or higher. The diagnosis and severity of GVHD were based on the clinical grading score [19,20]. Sinusoidal obstruction syndrome (SOS) was reported based on the clinical symptoms [21,22]. Causes of death were determined based on "the primary cause of death" reported by the attending physicians. When the primary cause of death was GVHD or multiorgan failure (MOF), the causes of death were divided into liver GVHD and GVHD without liver involvement and into MOF with hepatic failure and MOF without hepatic failure based on the secondary causes of death, respectively. Fatal hepatic problems were defined as SOS, liver GVHD, hepatic failure due to uncertain causes, and MOF with hepatic failure. Furthermore, the primary cause of death was replaced by "the secondary cause of death" when the secondary cause of death was "rejection," "relapse," or "secondary malignancy."

Statistical analysis

Categorical and continuous variables were compared using Fisher's exact test and the Mann-Whitney test, respectively. Relapse and nonrelapse mortality (NRM) were considered as competing risk events for each other. The probabilities of relapse and NRM were estimated by cumulative incidence functions, and differences between groups were qualified by Gray's method. The cumulative neutrophil and platelet recoveries and incidences of Grades 2 to 4 aGVHD and cGVHD were also estimated and compared by Gray's method considering death without these events as a competing risk. Overall survival (OS) was estimated by the Kaplan-Meir method and compared by a log-rank test. These probabilities were estimated with a 95% confidence interval (CI). In a multivariate analysis, the Cox proportional hazard model and Gray-Fine's methods were used for OS and the cumulative incidences of events other than OS, respectively, using the following variables: HCV serostatus, gender, age, disease, disease risk, performance status, the presence of prior autologous HCT, EBMT risk score, ABO match, sex match, HLA match, conditioning regimen, GVHD prophylaxis, and donor sources. The hazard ratio (HR) of HCV seropositivity was adjusted for variables with a P-value of less than 0.1 in a univariate analysis with stepwise deletions. While all of the eligible recipients were included in the analysis of neutrophil and platelet recovery, aGVHD, cGVHD, and OS, recipients who had received HCT in nonremission and had never achieved remission after HCT until the last observation were excluded from the analysis of NRM. The impact of HCV was also compared in three subgroups that were stratified according to the donor source: related donors, unrelated BMT, and unrelated CBT. Statistical significance was defined as a two-tailed P-value of less than 0.05. All data management and statistical calculations were performed using Stata version 12.0 and R version 2.13.0.

Results

Patient characteristics

Among the 7,831 recipients who received their first allogeneic HCT between 2006 and 2009, 136 HCV-positive patients were identified. Their characteristics are shown in Table I. The median age of HCV-positive and -negative patients was 49 (range: 18–73) years and 47 (range: 16–82) years, respectively. The HCV-positive group had higher proportions of male patients (67% vs. 59%, $P = 0.053$), female to male HCT (30% vs. 22%, $P = 0.080$), and tacrolimus-based GVHD prophylaxis (63% vs. 55%, $P = 0.076$), although the differences were not significant. There was no difference in other factors, including disease, disease risk, performance status, EBMT risk score, and conditioning regimens, between the two groups (Table I). Regarding infused cell doses, no differences were observed between the two groups when we analyzed according to donor sources: 2.7×10^8/kg vs. 2.6×10^8/kg total nuclear cells (TNC) in related BMT ($P = 0.29$), 3.5×10^6/kg vs. 3.7×10^6/kg CD34-positive cells in related PBSCT ($P = 0.66$), 2.6×10^8/kg TNC vs. 2.4×10^8/kg TNC in unrelated BMT ($P = 0.27$), and 0.24×10^8/kg vs. 0.25×10^8/kg TNC in each in the related CBT ($P = 0.83$).

Hematopoietic recovery

The cumulative probability of neutrophil recovery at 30 days after HCT in the HCV-positive group (79% [95% CI: 72–85]) tended to be lower than that in the HCV-negative group (87% [95% CI: 86–88]), but this difference was not significant ($P = 0.087$) (Fig. 2A). In subgroup analyses, the rates of neutrophil recovery at 30 days after HCT tended to be lower in the HCV-positive group in the unrelated BMT group (84% vs. 92%, $P = 0.094$) and significantly lower in the unrelated CBT group (46% vs. 68%, $P = 0.020$). On the