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Brief report

Successful sustained engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with severe aplastic anemia

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We retrospectively analyzed 12 consecutive adult severe aplastic anemia patients who received unrelated umbilical cord blood transplantation after a reduced-intensity conditioning regimen (RI-UCBT). The conditioning regimen consisted of 125 mg/m^2 fludarabine, 80 mg/m^2 melphalan, and 4 Gy of total body irradiation. The median infused total nucleated cell number and CD34$^+$ cell number were 2.50 × 10^7/kg and 0.76 × 10^5/kg, respectively. Eleven of the 12 patients achieved primary neutrophil and platelet engraftment. All patients who achieved engraftment had complete hematologic recovery with complete donor chimerism, except for one patient who developed late graft failure 3 years after RI-UCBT. Two of the 12 patients died of idiopathic pneumonia syndrome, and the remaining 10 patients are alive, having survived for a median of 36 months. Our encouraging results indicate that RI-UCBT may become a viable therapeutic option for adult severe aplastic anemia patients who lack suitable human leukocyte antigen-matched donors and fail immunosuppressive therapy. (*Blood.* 2011;117(11):3240-3242)

Introduction

Bone marrow transplantation from a human leukocyte antigen (HLA)-matched sibling is recommended as first-line therapy for younger patients with severe aplastic anemia (SAA).[1,2] However, many patients lack HLA-matched sibling donors. Bone marrow transplantation from an HLA-matched unrelated donor has been an alternative therapeutic option for patients who fail one or more courses of immunosuppressive therapy, but high rates of graft failure (GF), graft-versus-host disease (GVHD), and infection still remain to be solved.[3] The number of unrelated umbilical cord blood transplantations (UCBTs) has been increasing.[4] However, little information has been available on whether UCBT is feasible for SAA patients. We reported successful urgent UCBT using reduced-intensity (RI) conditioning for a 70-year-old SAA patient in 2003.[5] Here we present successful sustained engraftment of 11 consecutive patients with SAA who received RI-UCBT with the same RI conditioning regimen after the first report.

Methods

This study included 12 consecutive adult patients with acquired SAA who underwent RI-UCBT at our institute from September 2002 through January 2009. The patients' characteristics and umbilical cord blood (UCB) units are summarized in Table 1. Their median age was 49 years (range, 20-70 years). Four cases of severe, 6 of very severe, and 2 of fulminant type were included according to criteria as previously reported.[2,6] Fulminant type was defined as no neutrophils in the peripheral blood at diagnosis despite administration of granulocyte-colony stimulating factor. Ten patients, except for the 2 patients with fulminant type, had failed at least one course of immunosuppressive therapy. All patients gave their written informed consent in accordance with the Declaration of Helsinki, and the study was approved by the Toramon Hospital Institutional Review Board. UCB units were obtained from the Japanese Cord Blood Bank Network, and single UCB unit was infused in all the studied patients. All UCB units were serologically typed for HLA-A, -B, and -DR antigen before selection and were tested by high-resolution DNA typing before transplantation. The degree of mismatch is expressed using antigen level at HLA-A and -B, and allele level at DRB1. ABO incompatibility was not incorporated as one of the factors used in CB unit selection. The median total nucleated cell number and CD34$^+$ cell number at cryopreservation were 2.50 × 10^7/kg (range, 1.83-4.39 × 10^7/kg) and 0.76 × 10^5/kg (range, 0.27-1.52 × 10^5/kg), respectively. Anti-HLA antibodies were screened before transplantation in 6 patients using a FlowPRA method (One Lambda), and LAB Screen PRA or Single Antigen (One Lambda) was used to identify HLA antibody specificities.[7,8] All patients were conditioned with 25 mg/m^2 fludarabine daily for 5 days, 40 mg/m^2 melphalan daily for 2 days, and 4 Gy of total body irradiation in 2 fractions in 1 day. GVHD prophylaxis consisted of cyclosporine in 2, tacrolimus in 2, and tacrolimus plus mycophenolate mofetil in 8. Assessment of engraftment, GF, chimerism, GVHD, and supportive care during transplantation were performed as previously reported.[9,10] Karnofsky performance status score was assessed as surrogate for quality of life of the survivors. Overall survival was estimated using the Kaplan-Meier method.

Results and discussion

Patients' outcomes are summarized in Table 2. Eleven of the 12 patients achieved primary neutrophil and platelet engraftment. The median times to achieve neutrophil engraftment and platelet count more than 20 × 10^9/L were 18 days (range, 12-28 days) and

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Table 1. Characteristics of patient, grafts, and GVHD prophylaxis

Case no.	Age, y	Previous treatment	Interval from diagnosis to UCBT, mo	Previous transfusion times (RBCs/platelet)	Disease status at UCBT	HLA match	HLA Ab (reactive to CB)	ABO group (R/D)	TNC $\times 10^7$/kg	$CD34^+$, $\times 10^5$/kg	GVHD prophylaxis
1	70	CSA	3	11/14	SAA	4/6	NT	A/A	4.00	1.23	CSA
2	20	ATG + CSA	78	> 20/> 20	VSAA	4/6	NT	B/O	2.65	1.07	CSA
3	22	ATG + CSA, PSL	157	> 20/> 20	SAA	4/6	NT	A/O	2.26	0.27	Tac
4	26	ATG + CSA	3	> 20/> 20	VSAA	5/6	NT	A/A	2.65	0.70	Tac
5	59	ATG + CSA	8	> 20/> 20	SAA	5/6	Positive (no)	O/O	2.15	1.52	Tac + MMF
6	49	ATG + CSA, PSL	12	> 20/> 20	VSAA	3/6	NT	A/A	2.04	0.62	Tac + MMF
7	70	None	1	5/8	Fulminant	4/6	Positive (yes)	A/O	4.39	1.29	Tac + MMF
8	52	None	1	4/6	Fulminant	4/6	NT	AB/A	3.20	0.49	Tac + MMF
9	46	ATG + CSA	45	> 20/> 20	VSAA	4/6	Positive (no)	AB/O	1.83	0.42	Tac + MMF
10	49	ATG + CSA, PSL	327	> 20/> 20	VSAA	6/6	Positive (no)	B/O	2.34	0.82	Tac + MMF
11	65	CSA	6	16/> 20	VSAA	6/6	Positive (no)	A/A	3.31	0.56	Tac + MMF
12	31	ATG + CSA, PSL	215	> 20/> 20	SAA	4/6	Positive (no)	B/O	2.09	1.26	Tac + MMF

RBC indicates red blood cell; CB, cord blood; R, recipient; D, donor; TNC, total nucleated cells; CSA, cyclosporine-A; ATG, antithymocyte globulin; PSL, prednisone; VSAA, very severe aplastic anemia; NT, not tested; Tac, tacrolimus; and MMF, mycophenolate mofetil.

42 days (range, 26-64 days), respectively. All patients who achieved engraftment had complete hematologic recovery and were free from transfusion, and they showed complete donor chimerism at the time of the first chimerism analysis (median, 14 days; range, 11-73 days). One patient developed primary GF and was later found to have antibody against mismatched HLA on donor cells. Another patient developed secondary GF 3 years after UCBT. Both patients underwent a second RI-UCBT and obtained rapid donor engraftment. The negative impact of multiple transfusions before transplantation was not detected (Tables 1-2). Among 11 evaluable patients, 2 developed grade I and 5 developed grade II acute GVHD. Of the 9 patients who survived longer than 100 days after transplantation, 3 developed limited type of chronic GVHD. No patients developed grade III-IV acute GVHD and extensive type of chronic GVHD. Two of the 12 patients died of idiopathic pneumonia syndrome, and the remaining 10 patients are alive, having survived for a median of 36 months (range, 14-91 months). The probability of overall survival at 3 years was 83.3% (Figure 1). The surviving patients had high Karnofsky performance status score with a median of 90% (range, 60%-100%).

The present study demonstrated that our RI conditioning regimen allows a sufficient sustained engraftment of UCB in adult SAA patients. The RI conditioning regimen was originally developed in our institute for UCBT for various hematologic malignancies.[9] Eleven of the 12 patients achieved primary engraftment, which compares favorably with previously reported engraftment rates of UCBT for SAA.[11-16] Our RI conditioning regimen would be more potent than the others to overcome immunologic barriers for engraftment. Cell dose has been known to significantly influence the rate of engraftment after UCBT.[14] In the present study, although the cell dose was not very large, sufficient engraftment was seen. Any significant relationship between cell dose (total nucleated cell, ≥ 2.5 vs $< 2.5 \times 10^7$/kg; $CD34^+$, ≥ 0.8 vs $< 0.8 \times 10^5$/kg) and engraftment kinetics were observed (data not shown). Thus, not just cell dose but other factors, such as the intensity of the conditioning regimen and posttransplantation immunosuppression, may be important to achieve better engraftment after UCBT for SAA patients. Interestingly, all 6 patients who were screened for HLA antibodies before transplantation had HLA antibodies, and the one case who had positive HLA antibodies against an HLA on a transplanted UCB unit was the only one who failed primary engraftment. Recently, Takanashi et al reported that, in large number of UCBT for various hematologic malignancies, the

Table 2. Outcomes of 12 patients after reduced-intensity unrelated cord blood transplantation

Case no.	Days to ANC > 0.5 × 10^9/L	Days to PC > 20 × 10^9/L	% Donor chimerism (days tested, methods)	aGVHD	cGVHD	Discontinuation of IS (mo)	Complications	Survival (mo)
1	12	52	100 (14, FISH)	Grade II (skin)	No	Yes (3)	Possible IPA	Alive (91)
2	20	64	> 90 (49, PCR-STR)	Grade II (skin)	Limited	Yes (2)	No	Alive (90)
3	26	42	100 (26, FISH)	No	No	Yes (26)	Yes	Alive (69)
4	18	53	100 (18, FISH)	No	No	Yes (5)	*Pneumocystis jirovecii*, late GF, rescued by second RI-UCBT	Alive (69)
5	16	26	96.6 (14, FISH)	Grade I (skin)	Limited	Yes (14)	Norwalk virus colitis, EBV-PTLD	Alive (39)
6	28	64	99.6 (11, FISH)	No	NE	No	IPS	Dead; IPS (3)
7	No	No	48.8 (10, FISH), 4.3 (15, FISH)	NE	NE	NE	Primary GF, rescued by second RI-UCBT	Alive (32)
8	18	28	99.2 (13, FISH)	Grade II (skin, gut)	No	Yes (7)	CMV colitis, EBV-PTLD	Alive (28)
9	28	43	> 90 (14, PCR-STR)	Grade I (skin)	NE	No	HSV pneumonia, IPS	Dead; IPS (3)
10	15	27	99 (73, FISH)	No	Limited	No	No	Alive (22)
11	15	27	100 (20, FISH)	Grade II (skin, gut)	No	No	No	Alive (22)
12	13	28	100 (14, FISH)	Grade II (gut)	No	No	No	Alive (14)

ANC indicates absolute neutrophil count; PC, platelet count; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; IS, immunosuppressant; FISH, fluorescence in situ hybridization; PCR-STR, PCR of short tandem repeat; NE, not evaluable; IPA, invasive pulmonary aspergillosis; EBV-PTLD, Epstein-Barr virus–associated posttransplantation lymphoproliferative disorder; and IPS, idiopathic pneumonia syndrome.

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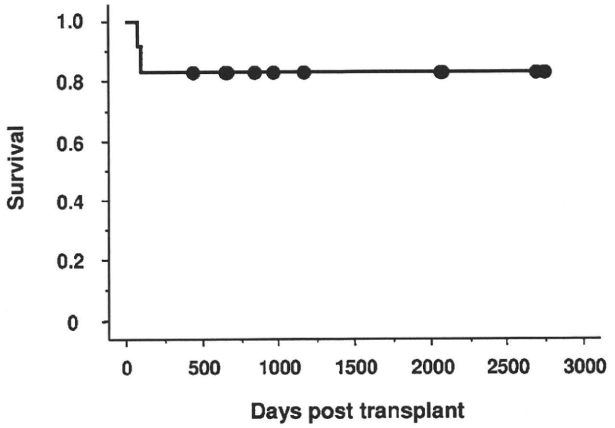

Figure 1. Survival of 12 patients with SAA undergoing unrelated cord blood transplantation.

patients with anti-HLA antibodies, when the specificity corresponding to mismatched antigen in UCB graft, showed significantly lower neutrophil or platelet recovery than those with antibody-negative or -positive but not corresponding to UCB graft.[17] Although the observations may differ from that of diverse populations and warrants further investigation, if possible, the use of a UCB unit with corresponding HLA antibodies in the recipient should be avoided.

Three-year survival in the studied patients was 83.3%. In addition to high rate of engraftment, the low risk of severe GVHD might contribute to high survival rate with good quality of life, and seems to be one of the important advantages of using a UCB unit for SAA patients. The other advantage of the use of UCB units is rapid availability. In the present study, 2 patients with fulminant type could be rescued by urgent hematopoietic stem cell transplantation using UCB units. More than 90% of recipients can find a suitable UCB unit in Japan; thus, UCB expands the chance to receive transplantation for those who need it urgently.

In conclusion, this retrospective study strongly suggests the feasibility and effectiveness of RI-UCBT for adult SAA patients. RI-UCBT may become a viable therapeutic option for those who lack suitable HLA-matched donors and who fail or relapse after immunosuppressive therapy. Although our results should be interpreted with caution because of the small number of patients and still short follow-up duration, we think that RI-UCBT with the conditioning regimen presented here deserves further evaluation in a prospective trial, hopefully in a multicenter setting.

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Authorship

Contribution: H.Y. and D.K. performed transplantation, analyzed extracted data, and contributed to writing the paper; A.Y. reviewed histopathologic sections; H.Y. and N.M. performed statistical analysis; N.U., K. Izutsu, and S. Taniguchi reviewed study design and methods; and K. Ishiwata, H.A., S. Takagi, M.T., N.N., Y.A.-M., K.M., A.W., and S.M. performed transplantation and contributed to writing the paper.

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Successful engraftment after reduced-intensity umbilical cord blood transplantation for myelofibrosis

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Brief report

Successful engraftment after reduced-intensity umbilical cord blood transplantation for myelofibrosis

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Although allogeneic hematopoietic stem cell transplantation has recently been applied to patients with myelofibrosis with reproducible engraftment and resolution of marrow fibrosis, no data describe the outcomes of umbilical cord blood transplantation. We describe 14 patients with primary (n = 1) and secondary myelofibrosis (n = 13) who underwent reduced-intensity umbilical cord blood transplantation. Conditioning regimens included fludarabine and graft-versus-host disease prophylaxis composed cyclosporine/tacrolimus alone (n = 6) or a combination of tacrolimus and mycophenolate mofetil (n = 8). Thirteen patients achieved neutrophil engraftment at a median of 23 days. The cumulative incidence of neutrophil and platelet engraftment was 92.9% at day 60 and 42.9% at day 100, respectively. Posttransplantation chimerism analysis showed full donor type in all patients at a median of 14 days. The use of umbilical cord blood could be feasible even for patients with severe marrow fibrosis, from the viewpoint of donor cell engraftment. (*Blood*. 2010;116(4):649-652)

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered the only curative therapy for primary myelofibrosis (MF) and MF secondary to hematologic malignancies.[1] Myeloablative conditioning regimens are associated with high rates of transplantation-related mortality (TRM), especially among elderly patients.[2-4] Recent reports indicate that reduced-intensity conditioning (RIC) regimens can improve outcomes in such patients.[5-8] These reports also confirm the safety and effectiveness of bone marrow (BM) and mobilized peripheral blood stem cells (PBSCs) from matched related or unrelated donors as stem cell sources. In contrast, the feasibility of umbilical cord blood transplantation (CBT) for MF is unknown.

CBT is a valuable alternative to allo-HSCT for treating patients with hematologic diseases who do not have matched related or unrelated donors and who need urgent transplantation.[9-12] On the other hand, engraftment delay or failure is one of the most critical issues that can arise after CBT. The limited doses of total nucleated cells and $CD34^+$ cells in umbilical cord blood and a human leukocyte antigen (HLA) disparity influence the kinetics of hematopoietic recovery.[13-15] Considering these disadvantages of CBT, delayed engraftment or engraftment failure is a great concern for MF patients who undergo CBT.[16] The goal of this study is to evaluate the feasibility of reduced-intensity CBT (RI-CBT) for MF.

Methods

The records of all patients who underwent RI-CBT at Toranomon Hospital from August 2003 and December 2008 were reviewed to identify patients who had histologically confirmed MF before starting the conditioning regimen. Marrow fibrosis was assessed on silver-stained BM trephine biopsies and classified into 4 grades according to the World Health Organization classification.[17] All the patients were incurable using conventional approaches and lacked an HLA-identical sibling or a suitable unrelated donor from the Japan Marrow Donor Program. Cord blood units serologically matching more than or equal to 4 of 6 HLA antigens and containing at least 1.8×10^7 nucleated cells/kg of recipient body weight before freezing were obtained from the Japan Cord Blood Bank Network. Conditioning regimens were determined at the discretion of each physician according to the patients' disease, disease status, and history of prior therapy. Information about baseline demographics, clinical characteristics, transplantation, and its outcome were collected from medical records. Assessment of engraftment, chimerism (one or more times a week), pre-engraftment immune reactions, graft-versus-host disease (GVHD), and supportive care during transplantation were performed as previously reported.[18-20] Cumulative incidences were estimated for neutrophil and platelet engraftment. Overall survival was estimated using the Kaplan-Meier method, taking the interval from date of transplantation to death or last contact.[21] The Institutional Review Board of Toranomon Hospital approved the study, and written informed consent was provided by all patients to use their records in accordance with the Declaration of Helsinki.

Results and discussion

Fourteen MF patients (median age, 57.5 years; range, 46-72 years) were extracted. Table 1 shows the clinical characteristics of the patients. They had primary MF (n = 1), leukemic transformation from MF secondary to polycythemia vera or essential thrombocytosis (n = 2), or MF secondary to acute myeloid leukemia (AML; n = 11; AML with multilineage dysplasia in all patients except for one with de novo AML). All but one patient had the highest-grade

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Table 1. Patient characteristics

Patient no.	Age, y/sex	Diagnosis	Disease status	Time from diagnosis to transplantation, d	Pretransplantation MF grade	Splenomegaly	Cytogenetics
1	55/M	AML/MF/ET	PIF	1732	3	Yes	Normal
2	53/M	PMF	Untreated	307	3	Yes	NA
3	61/M	AML/MDS	PIF	116	3	Yes	Complex*
4	51/F	AML/MDS	PIF	740	3	Yes	Normal
5	61/F	AML/MDS	PIF	227	3	Yes	NA
6	55/M	AML/MDS	Untreated	299	3	Yes	Complex
7	46/M	AML/MDS	Untreated	600	3	Yes	NA
8	58/M	AML/MDS	Untreated	544	3	Yes	Complex
9	67/F	AML/MF/PV	Untreated	150	3	Yes	t(3;3)(q21;q26), −7
10	53/M	De novo AML	PIF	111	3	No	Complex
11	57/F	AML/MDS	Untreated	352	3	Yes	Complex with t(9;22)(q34;q11)
12	62/M	AML/MDS	Untreated	147	3	Yes	add(1)(p32), −7
13	72/F	AML/MDS	PIF	329	2	No	Complex with t(9;22)(q34;q11)
14	66/M	AML/MDS	Untreated	92	3	No	Normal

AML indicate acute myeloid leukemia; MF, myelofibrosis; ET, essential thrombocythemia; PIF, primary induction failure; PMF, primary myelofibrosis; AML/MDS, acute myeloid leukemia with multilineage dysplasia; NA, not available; and PV, polycythemia vera.

*Complex karyotype was defined as 3 or more abnormalities at pretransplantation evaluation.

MF. The median time from diagnosis to transplantation was 303 days (range, 92-1732 days). Table 2 shows the transplantation characteristics. All received purine analog–based conditioning regimens composing fludarabine phosphate (125-180 mg/m^2), melphalan (80-140 mg/m^2), or intravenous busulfan (12.8 mg/kg) and 0 to 8 Gy of total body irradiation. GVHD prophylaxis included tacrolimus and mycophenolate mofetil for 8 patients, tacrolimus, or cyclosporine A alone in 6. Neutrophil and platelet engraftment was achieved in 13 and 6 patients, respectively, of the 14 patients. The median time to engraftment was 23 days (range, 14-43 days) and 53 days (range, 44-102 days) for neutrophils and platelets, respectively. The cumulative incidence of neutrophil engraftment at day 60 and platelet engraftment at day 100 was 92.9% and 42.9%, respectively (Figure 1A-B). Chimerism analysis of the peripheral blood of 8 patients and the BM of 6 showed that donor chimerism was complete (donor > 90%) in all of them. The median length of time required to achieve complete donor chimerism was 14 days (range, 7-33 days; Figure 1A). Of the 14 patients, 9 (64%) developed pre-engraftment immune reactions. Five (36%) developed acute GVHD grades 2 to 4. No extensive chronic GVHD was observed in 6 evaluable patients (Table 3). Five patients remained alive at last contact, representing an estimated probability of overall survival of 28.6% at 4 years (Figure 1C). All the patients who could not achieve platelet engraftment died, whereas 4 of 7 patients (57%) who achieved platelet engraftment survived. In 9 patients who died after RI-CBT, 5 patients died of relapsed leukemia. Non–relapse-related causes of death composed infection (n = 2), GVHD (n = 1), and multiple organ failure (n = 1). Marrow fibrosis disappeared in 2 evaluated patients who survived beyond 100 days.

This study demonstrated that umbilical cord blood results in successful engraftment, even for patients with severe marrow fibrosis in the setting of the RIC regimen, which was similar to that of other stem cell sources, such as BM and PBSCs.[2-8,22] Although marrow fibrosis has historically been considered as a relative contraindication to transplantation because of concerns over an insufficient and/or dysfunctional niche in which allogeneic hematopoietic stem cell engraftment may proceed, recent outcomes of allo-HSCT for MF support the concept that marrow fibrosis is not an absolute barrier to allogeneic hematopoietic stem cell engraftment.[1] However, data from these reports are limited to transplantations with BM and PBSCs, and no information is available about umbilical cord blood. Delayed hematopoietic recovery and low engraftment rate, perhaps because of limited infused cell doses and

Table 2. Transplantation characteristics

Patient no.	TNC, ×10^7/kg	CD34$^+$, ×10^5/kg	Sex match	HLA match	Blood type match	Conditioning regimen	GVHD prophylaxis
1	2.52	0.823	MM	4/6	MM	F125/M80/TBI4	CsA
2	2.62	0.678	MM	4/6	MM	F125/M80/TBI4 + SRT	TAC
3	3.17	1.60	Match	4/6	Match	F125/M80/TBI4	TAC
4	2.43	NA	MM	4/6	Match	F125/M80/TBI4	TAC
5	3.94	2.26	MM	5/6	Match	F180/M140	TAC/MMF
6	2.31	0.887	MM	4/6	MM	F125/M80/TBI4	TAC
7	2.72	1.03	Match	4/6	MM	F125/Mel140/TBI4	TAC/MMF
8	2.46	0.773	MM	4/6	Match	F180/M140	TAC
9	1.99	1.24	MM	4/6	MM	F125/M80/TBI4 + SRT	TAC/MMF
10	3.25	0.547	MM	4/6	Match	F125/M140/TBI4	TAC/MMF
11	3.31	1.31	MM	4/6	Match	F125/M80/TBI8	TAC/MMF
12	2.37	0.873	MM	4/6	MM	F125/M80/TBI8	TAC/MMF
13	2.51	0.993	MM	4/6	Match	Flu180/B12.8/TBI2	TAC/MMF
14	2.50	0.554	MM	5/6	Match	F125/M120	TAC/MMF

TNC indicates total nucleated cell count; MM, mismatch; F, fludarabine (mg/m^2); M, melphalan (mg/m^2); TBI, total body irradiation; CsA, cyclosporine; SRT, splenic radiation; TAC, tacrolimus; MMF, mycophenolate mofetil; and B, intravenous busulfan (mg/kg).

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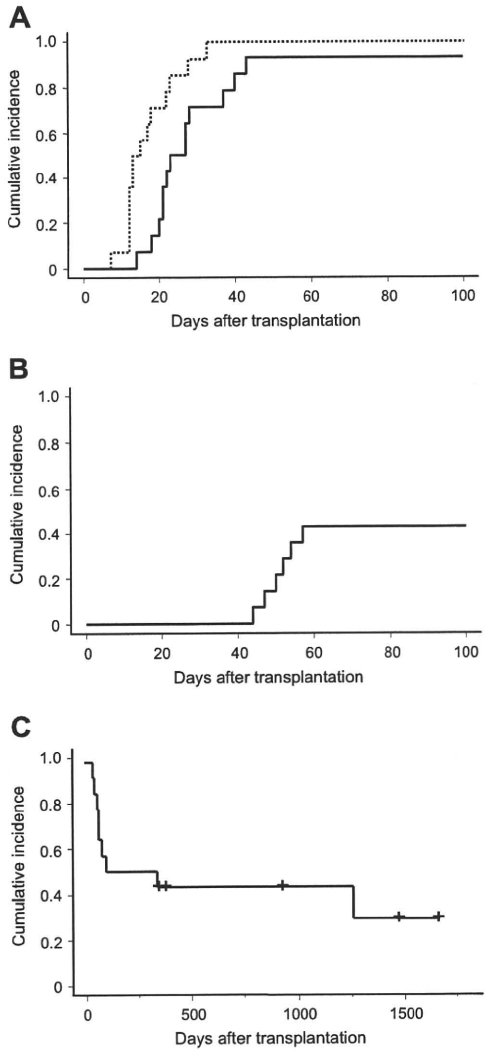

Figure 1. Cumulative incidence of engraftment. (A) Solid and broken lines indicate cumulative incidence of neutrophil engraftment and complete donor chimerism, respectively. (B) Cumulative incidence of platelet engraftment. (C) Overall survival.

HLA disparities, might limit the use of umbilical cord blood in these cases.[13-15,19] However, the present study demonstrated an equivalent or superior engraftment rate after CBT compared with allo-HSCT using other stem cell sources.[1-8] We also confirmed an early chimerism switching in the present study. All 14 patients achieved complete donor chimerism at a median of 14 days, which was much earlier than that with neutrophil engraftment. Moreover, we histologically confirmed that RI-CBT had the potential to cure marrow fibrosis in 2 evaluated patients. These data suggest that RI-CBT is an encouraging strategy for treating MF.

Despite successful engraftment, overall survival was poor in the present study compared with previous reports. However, this result does not eliminate the feasibility of RI-CBT for MF patients. Our patient series included only one primary MF. In 13 of 14 patients, MF coexisted with AML simultaneously. High prevalence of concurrent AML with MF in the present study probably made overall survival poorer. However, MF with AML is also challenging issues in real clinical settings. Physicians occasionally face rapidly growing AML cases with concurrent marrow fibrosis, especially in the elderly, for whom urgent allo-HSCT is the only curative therapy. For those patients, CBT is attractive because of its accessibility. In this viewpoint, we think that the feasibility of RI-CBT suggested in the present study is encouraging.

In conclusion, our data suggest that RI-CBT is feasible, even for patients with severe marrow fibrosis, from the viewpoint of donor cell engraftment. Especially for MF with AML, further improvements are required in the next place to overcome poor survival resulting from relapse.

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Table 3. Outcome of RI-CBT

Patient no.	Neutrophil engraftment, d	Platelet engraftment, d	Pre-engraftment immune reactions*	aGVHD 2-4	aGVHD 3-4	cGVHD	Survival	Survival from transplantation, d	Cause of death
1	27	52	No	Yes	No	No	Dead	1264	Relapse
2	22	54	Yes	No	No	NE	Alive	1672	NA
3	23	Not engrafted	Yes	Yes	Yes	NE	Dead	68	Infection
4	40	102	Yes	Yes	No	Limited	Alive	1481	NA
5	18	44	Yes	No	No	No	Dead	344	Relapse
6	14	Not engrafted	Yes	No	No	NE	Dead	78	Relapse
7	21	57	Yes	Yes	Yes	Limited	Alive	937	NA
8	Not engrafted	Not engrafted	No	No	No	NE	Dead	42	Infection
9	37	Not engrafted	Yes	No	No	NE	Dead	45	MOF
10	28	Not engrafted	Yes	Yes	Yes	NE	Dead	64	GVHD
11	27	Not engrafted	Yes	No	No	NE	Dead	61	Relapse
12	43	NA	No	No	No	Limited	Alive	392	NA
13	21	47	No	No	No	Limited	Alive	355	NA
14	20	50	No	No	No	NE	Dead	100	Relapse

aGVHD indicates acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; NE, not evaluable; NA, not applicable; and MOF, multiple organ failure.

*Pre-engraftment immune reactions were diagnosed when febrile patients developed skin eruption, diarrhea, jaundice, or body weight gain of more than 10% of baseline, with no direct evidence of infection or adverse effects of medication, developing more than 6 days before engraftment.[18]

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Authorship

Contribution: S. Takagi performed transplantation, analyzed extracted data, and contributed to writing the paper; Y.O. analyzed histologic sections; N.U., K.T., K.I., M.T., H.Y., Y.A.-M., K.M., A.W., and S.M. performed transplantation and contributed to writing the paper; N.M. performed transplantation and supported statistical analysis; K.O. reviewed histologic sections and contributed to writing the paper; and S. Taniguchi reviewed the study method and organized this study.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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LETTER TO THE EDITOR

T-cell post-transplant lymphoproliferative disorder in a patient with chronic idiopathic myelofibrosis following allogeneic PBSC transplantation

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Post-transplant lymphoproliferative disorder (PTLD) is a well-recognized complication after solid organ and hematopoietic SCTs (HSCTs). The majority are of B-cell origin and EBV related.[1] Most of the T-cell PTLD cases have been described as occurring after solid organ transplantations;[2] T-cell PTLD cases following HSCT are exceedingly rare. There are only three reported cases of T-cell PTLD following allogeneic HSCT[3] and four cases following autologous HSCT.[4–7] Here we report a case of T-cell PTLD after allogeneic-PBSC transplantation (allo-PBSCT) in a patient with chronic idiopathic myelofibrosis (CIMF).

A 44-year-old Japanese woman with anemia and fever was diagnosed with CIMF in November 2006. At the time of her diagnosis, her WBC count was 900/μl, Hb 6.9 g/dl, plt count 39 000/μl with no morphologically abnormal cells in her peripheral blood, and an abdominal CT scan showed mild splenomegaly without hepatomegaly, lymphadenopathy or liver tumor. A specimen of her biopsied BM showed diffuse fibrosis and a decreased number of hematopoietic cells. No abnormal cell proliferation was observed. In December 2006, she underwent allo-PBSCT from an HLA-identical brother. Neutrophil engraftment was achieved on day 17 after transplant, and BM analysis showed full hematological recovery with 100% donor-type chimerism assessed by Y chromosome-based FISH analysis. As grade II acute GVHD involving the skin and subsequently an extensive type of chronic GVHD (cGVHD) developed; continued immunosuppressive therapy with cyclosporine and prednisolone was required for several months after the transplant. At 5 months after transplant, a liver tumor, 2 cm in diameter, was detected by an abdominal CT scan. Although PTLD was raised as a differential diagnosis, biopsied liver tissue was inadequate for pathological examination. Immunosuppressive therapy was reduced, resulting in a decrease in liver tumor size to 1.6 cm in 2 months. However, a subsequent flare-up of cGVHD required more intensive immunosuppressive therapy, and the liver tumor's diameter increased twice in size. A liver tumor biopsy performed at this time showed a diffuse proliferation of atypical lymphoid cells (Figure 1a). Immunohistochemically, these tumor cells were positive for LCA, CD3, CD7 and CD8, and negative for CD4, CD5, CD34, CD79a, MPO, CD30, CD56 and TdT (Figure 1b). These pathological findings are compatible with peripheral T-cell lymphoma-unspecified (Figure 1c). EBV infection was not detected by *in situ* hybridization. Y chromosome-based FISH analysis revealed the tumor cells were of recipient origin. She suffered from fever, pancytopenia and decreased liver function, and was hospitalized for further therapy in November 2007. BM examination showed infiltration of 4% abnormal lymphoid cells and the proliferation of macrophage with hemophagocytosis, with no sign of CIMF recurrence. Chromosome analysis of the BM cells showed 44, X, der(X)t(X;7)(q13;q11.2), add(2)(q21), add(4)(p11), add(4)(p16), der(9;17)(q10;q10), −10, −13, add(15)(p11), +mar [2/20]. An abdominal CT scan showed that the liver tumor grew rapidly to a size of $12 \times 6\,cm^2$ (Figure 1d). Serological tests for HIV, HBV, HCV and HTLV-1 were negative, and the EBV VCA IgG was positive but negative for IgM. Analyses by real-time PCR were negative for human herpesvirus-6, VZV, CMV and EBV in her peripheral blood. She was diagnosed with T-cell PTLD with lymphoma-associated hemophagocytic syndrome. CHOP therapy was started, but the disease progressed within 2 weeks after this. She underwent urgent unrelated cord blood transplantation (UCBT) from an HLA two antigen-mismatched donor. Her post-transplant course was complicated by sepsis, renal failure and respiratory failure. She died on day 6 after UCBT. An autopsy was not performed.

To our knowledge, there have been only four cases of T-cell PTLD following allo-SCT, including our case (Table 1). Time to T-cell PTLD diagnosis ranges from 2 to 43 months after a transplant. Although the type of PTLD was not consistent, ranging from precursor to peripheral T-cell neoplasms, none of them were associated with EBV infection. Our case was negative for EBV, and the type was peripheral T-cell lymphoma-unspecified.

There have been a few reports describing myelofibrosis in association with T-cell lymphoma.[8] In these cases, PDGF and tumor growth factor β, which may have been secreted by neoplastic T lymphocytes, had an important role in the development of myelofibrosis. In our case, there was no clinical evidence of T-cell lymphoma at the time of CIMF diagnosis, and no sign of myelofibrosis recurrence at the onset of T-cell lymphoma. Thus, the development of T-cell lymphoma in this case was considered to be independent of the CIMF.

All three patients reported as having T-cell PTLD following allo-SCT had severe GVHD and received a heavy dose of immunosuppressive agents, suggesting some viral agents in an immunosuppressed state may have an important role in the development of T-cell PTLD. However, we were unable to find any evidence of viral

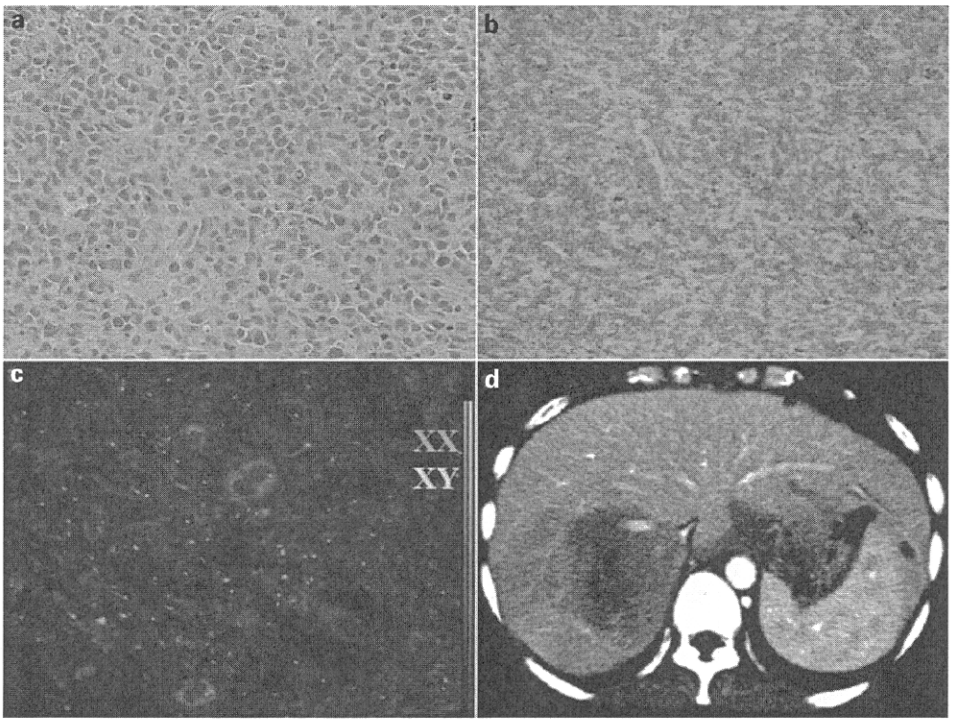

Figure 1 (**a**) Liver tumor biopsy shows monotonous infiltration of atypical lymphoid cells (H&E stain ×400). (**b**) Immunostaining for CD3 shows a large number of positive cells within the tumor. (**c**) Y chromosome-based FISH reveals the tumor cells are of recipient origin (XX signal). (**d**) Abdominal CT scan shows a low-density area with 12 cm diameter on the right side of the liver.

Table 1 T-cell post-transplant lymphoproliferative disorder after allogeneic stem cell transplantation

Authors	*Age/sex*	*Initial Dx*	*HSCT*	*Type of PTLD Dx (months after HSCT)*	*Origin*	*EBV*	*GVHD*	*Outcome (months after Dx)*
Zutter *et al.*[3]	14/M	AML	HLA-identical BM graft	T-lymphoblastic lymphoma (43)	Recipient	Neg	Mild aGVHD(S,L,Gut) Severe cGVHD(S,L,Gut)	Death (28)
	9/M	ALL	HLA-identical BM graft	T-lymphoblastic lymphoma (21)	Donor	Neg	Mild aGVHD(S) Severe cGVHD(S,L)	Death (6)
	2/F	ALL	HLA-2 mismatched BM graft	Polymorphic T-cell lymphoma (2)	Donor?	Neg	Severe aGVHD(S,L)	Death (0)
Present case	44/F	CIMF	HLA-identical allogeneic PBSC	PTCL-u (5)	Recipient	Neg	aGVHDII(S3,L0,Gut0) Extensive cGVHD(S,L)	Death (2)

Abbreviations: aGVHD = acute GVHD; cGVHD = chronic GVHD; CIMF = chronic idiopathic myelofibrosis; Dx = diagnosis; F = female; Gut = gastrointestinal tract; HSCT = hematopoietic stem cell transplantation; L = liver; M = male; neg = negative; PTCL-u = peripheral T-cell lymphoma-unspecified; PTLD = post-transplant lymphoproliferative disorder; S = skin.

infection and reactivation in our case and previously reported cases. It has been reported that only 15 of 76 cases of T-cell PTLD after solid organ transplantation were EBV positive,[9] and any other viral involvement has not been clearly demonstrated. These findings suggest that not only viral infection but also other factors, such as chronic antigenic stimulation, impaired immunoregulation and genetic factors, may be associated with the development of T-cell PTLD.[10]

The outcomes of reported T-cell PTLD so far are poor. All patients died because of the progression of the disease. In our patient, a transient response was observed by reducing immunosuppression, suggesting a graft-versus-lymphoma effect, which was necessitated to increase the immunosuppression. Standard cytotoxic chemotherapy led to a poor response in our patient, similar to the other cases previously described. More intensive chemotherapy, donor lymphocyte infusion or second HSCT should be considered at an early stage of the disease.

In conclusion, T-cell PTLD rarely occurs after allo-HSCT. Further research, however, is needed to fully characterize the clinicopathological features of this condition and to investigate the optimal therapy.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Pre-transplant imatinib-based therapy improves the outcome of allogeneic hematopoietic stem cell transplantation for *BCR–ABL*-positive acute lymphoblastic leukemia

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A high complete remission (CR) rate has been reported in newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph + ALL) following imatinib-based therapy. However, the overall effect of imatinib on the outcomes of allogeneic hematopoietic stem cell transplantation (allo-HSCT) is undetermined. Between 2002 and 2005, 100 newly diagnosed adult patients with Ph + ALL were registered to a phase II study of imatinib-combined chemotherapy (Japan Adult Leukemia Study Group Ph + ALL202 study) and 97 patients achieved CR. We compared clinical outcomes of 51 patients who received allo-HSCT in their first CR (imatinib cohort) with those of 122 historical control patients in the pre-imatinib era (pre-imatinib cohort). The probability of overall survival at 3 years after allo-HSCT was 65% (95% confidence interval (CI), 49–78%) for the imatinib cohort and 44% (95% CI, 35–52%) for the pre-imatinib cohort. Multivariate analysis confirmed that this difference was statistically significant (adjusted hazard ratio, 0.44, $P = 0.005$). Favorable outcomes of the imatinib cohort were also observed for disease-free survival ($P = 0.007$) and relapse ($P = 0.002$), but not for non-relapse mortality ($P = 0.265$). Imatinib-based therapy is a potentially useful strategy for newly diagnosed patients with Ph + ALL, not only providing them more chance to receive allo-HSCT, but also improving the outcome of allo-HSCT.
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Keywords: Philadelphia chromosome-positive acute lymphoblastic leukemia; imatinib; allogeneic hematopoietic stem cell transplantation

Introduction

The Philadelphia chromosome (Ph) presents in 20–25% of adult patients with acute lymphoblastic leukemia (ALL) and is an extremely unfavorable prognostic factor. The outcome of patients with Ph-positive ALL (Ph + ALL) following conventional chemotherapy is dismal, showing <20% long-term survival.[1–4] Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) has offered a curative option in Ph + ALL,[3–5] relatively high rates of relapse and non-relapse mortality (NRM) impair the treatment success even after allo-HSCT. The International Bone Marrow Transplant Registry reported a leukemia-free survival rate of 38% following human leukocyte antigen (HLA)-identical allo-HSCT for Ph + ALL patients transplanted in the first complete remission (CR).[6] Previously, we and others reported that imatinib-based chemotherapy produced very high CR rate, thus allowing a high proportion of patients to prepare for allo-HSCT.[7,8] However, because of the short observation period, the impact of imatinib-based therapy upon the survival outcomes after allo-HSCT remains unclear. To address whether allo-HSCT after imatinib-based therapy is a superior treatment approach to that after conventional chemotherapy, we conducted a retrospective analysis of Ph + ALL patients who underwent allo-HSCT before and after imatinib became available, using data from the Japan Adult Leukemia Study Group (JALSG) Ph + ALL202 study and from the nationwide database of the Japan Society of Hematopoietic Stem-cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP).

Patients and methods

Data source and patient selection criteria

We compared the transplantation outcome of patients treated by the JALSG Ph + ALL 202 study (imatinib cohort) with those in the historical control data in the pre-imatinib era from the JSHCT and JMDP (pre-imatinib cohort), in which information on patient survival, disease status and long-term complications, including chronic graft-versus-host disease (cGVHD) and second malignancies, is renewed annually using follow-up forms.[9,10] To

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attain an adequate level of comparability in terms of allo-HSCT, patients were selected according to the following criteria: (1) patients with *de novo* Ph+ALL; (2) age range of 15–65 years and (3) allo-HSCT during their first CR. A total of 122 patients who received allo-HSCT between January 1995 and December 2001 (before the approval of imatinib by the Japanese government) were selected. This study period of the pre-imatinib cohort included the pioneering period of cord blood transplantation (CBT) when the relevance of cell dose and HLA matching had not yet been recognized. Thus, the subjects were limited to those who received bone marrow (BM) or peripheral blood (PB) as a treatment graft.

Patients

Between September 2002 and May 2005, 100 newly diagnosed patients with Ph+ALL were registered to the JALSG Ph+ALL202 study, and received a phase 2 imatinib-combined chemotherapy as described previously.[7] Ph+ALL was diagnosed by the presence of Ph through chromosome and/or FISH analysis, and positivity for *BCR–ABL* fusion transcripts detection by real-time quantitative polymerase chain reaction (RQ–PCR) analysis.

Of 97 patients who achieved CR, 60 patients received allo-HSCT in their first CR. Of these 60 patients, 9 patients who received unrelated CBT were excluded in this analysis because of the reason as described at the selection criteria for control patients in the pre-imatinib era. Thus, 51 patients transplanted between February 2003 and December 2005 were analyzed. In the JALSG Ph+ALL202 study, allo-HSCT was recommended after achieving CR if an HLA-identical donor was available. The stem cell source for allo-HSCT was chosen in the following order: (1) matched-related allo-HSCT; (2) HLA-A, B and DRB1 allele matched (6/6) or DRB1 one-allele mismatched-unrelated allo-BMT, if patients had no HLA-matched-related donor and (3) unrelated CBT or HLA-mismatched-related allo-HSCT, if they had no donors described in (1) and (2). A prophylaxis for GVHD was determined by each institute, but did not include T-cell depletion. The study was approved by the institutional review board of each participating center and conducted in accordance with the Declaration of Helsinki.

Definition of engraftment and GVHD

Engraftment day was defined as the first day of three consecutive days when the absolute neutrophil count was $\geqslant 0.5 \times 10^9$/l. Graft failure was defined as the lack of any sign of neutrophil recovery. Engraftment that occurred after day 60 was also considered to be a graft failure. Patients who died early (<day 29) were excluded from the analysis of engraftment. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined according to previously described standard criteria.[11]

Quantitation of BCR–ABL transcripts

The copy number of *BCR–ABL* transcripts in BM was determined at a central laboratory using the RQ–PCR as described previously.[7] To minimize the variability in the results because of differences in the efficiency of cDNA synthesis and RNA integrity among the patient samples, the copy number of the *BCR–ABL* transcripts was converted to molecules per microgram RNA after being normalized by means of *GAPDH*. The normalized values of the *BCR–ABL* copies in each sample were reported as *BCR–ABL* number of copies. At least 5.7×10^5 copies/μg RNA *GAPDH* levels were required in a sample to consider a negative PCR result valid; otherwise, the sample was not useful for minimal residual studies. The threshold for quantification was 50 copies/μg RNA. The levels below this threshold were designated as 'not detected' or '<50 copies/μg'. In this study, the former was categorized as PCR negativity.

Minimal residual disease (MRD) at the time of HSCT was evaluated by the result of RQ–PCR within 30 days prior to transplantation.

Statistical considerations

The primary end point of this study was overall survival (OS) after allo-HSCT. Secondary end points included disease-free survival (DFS) and the incidence of aGVHD, cGVHD, NRM and relapse. We defined DFS events as relapse or death, whichever occurred earlier. The observation periods for OS were calculated from the date of transplantation until the date of the event or last known date of follow-up. The probabilities of OS and DFS were estimated using the Kaplan–Meier product limit method. The cumulative incidences of NRM, relapse, aGVHD and cGVHD were estimated as described elsewhere, taking the competing risk into account.[12] In each estimation of the cumulative incidence of an event, death without an event was defined as a competing risk. Risk factors for OS and DFS were evaluated by a combination of uni- and multivariate analyses. The following variables were evaluated for each analysis: imatinib-based therapy prior to HSCT, age group (under 40 versus 40 to 54 versus 55 and older), stem cell source (BM versus PB), HLA disparity (matched (HLA-identical siblings or 6/6 allele matched unrelated) versus mismatched), duration from diagnosis to HSCT and cGVHD as time-varying covariate (yes versus no). Univariate analysis was performed using Cox regression models or log-rank test. Multivariate analysis was performed using Cox proportional hazards regression model or competing risk regression model[13] as appropriate. For the evaluation of time-varying events, such as aGVHD or cGVHD, upon clinical outcomes, we treated these as time-varying covariates. Differences among groups in terms of demographic characteristics were tested using the χ^2 or Mann–Whitney tests as appropriate. All statistical analyses were conducted using STATA 11 (STATA Corp., College Station, TX, USA).

Results

Patient characteristics

In the imatinib cohort, there were 29 males and 22 females, with a median age of 38 years (range, 15–64 years). Regarding transcript types, 36 patients had minor *BCR–ABL* and 15 had major *BCR–ABL*. In 5 patients, pre-treatment cytogenetic data were not available, and of the remaining 46 patients, 8 showed t(9;22) only, 36 had additional chromosome aberrations and 2 showed normal karyotype. Of 48 patients who were evaluable for MRD analysis, 36 patients achieved PCR negativity at the time of HSCT.

Some of the clinical and biological features (such as presence of additional chromosome aberrations, *BCR–ABL* subtype, MRD status at HSCT and performance status at HSCT) were not available in the pre-imatinib cohort and not included in the present analysis.

Table 1 lists the characteristics of patients included in this comparative analysis. Some of the clinical features were significantly different between two cohorts: age distribution at HSCT ($P=0.048$), conditioning regimens ($P<0.001$), GVHD prophylaxis ($P<0.001$) and duration from diagnosis to HSCT ($P=0.041$). The majority of patients received the preparatory

Table 1 Patient characteristics ($N=173$)

Characteristic	*Imatinib cohort*	*Pre-imatinib cohort*	P
No. of transplantations	51	122	
Age, n (%)			0.048
–39	27 (53)	71 (58)	
40–54	17 (33)	49 (40)	
55–	7 (14)	2 (2)	
Median (range)	38 (15–64)	38 (15–57)	
Gender (male/female)	29/22	73/49	0.717
HSCT donor, n *(%)*			0.460
Related	24 (47)	73 (60)	
Unrelated	21 (41)	43 (35)	
HLA-mismatched related	6 (12)	6 (5)	
Hematopoietic cell source, n *(%)*			0.246
Bone marrow	35 (69)	94 (77)	
Peripheral blood	16 (31)	28 (23)	
Conditioning regimen, n *(%)*			<0.001
CY+TBI	24 (47)	26 (22)	
CY+CA+TBI	14 (27)	37 (31)	
CY+VP+TBI	2 (4)	21 (17)	
CY+TESPA+TBI	—	7 (6)	
CY+BU+TBI	—	6 (5)	
Flu+BU	3 (6)	—	
Flu+ LPAM ± TBI	2 (4)	—	
Others	6 (12)	25 (20)	
GVHD prophylaxis, n *(%)*			<0.001
Cyclosporine + sMTX	24 (47)	95 (80)	
Cyclosporine ± other	3 (6)	3 (2)	
Tacrolimus + sMTX	22 (43)	17 (14)	
Tacrolimus + other	—	4 (3)	
Median days from diagnosis to HSCT (range)	162 (67–512)	182 (66–834)	0.041

Abbreviations: BU, oral busulfan; CA, cytarabine; CY, cyclophosphamide; Flu, fuludarabine; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; LPAM, melphalan; sMTX, short-term methotrexate; TBI, total body irradiation; TESPA, tespamine; VP, etoposide.

regimen of total body irradiation followed by cyclophosphamide and/or cytarabine. Five patients aged >55 in the imatinib cohort were given a reduced intensity regimen consisting of fludarabine and melphalan or busulfan. In the pre-imatinib cohort, a combination of cyclosporine (CsA) and short-term methotrexate (sMTX) was mostly used in the prophylaxis of GVHD. On the other hand, both CsA + sMTX and tacrolimus (FK506) + sMTX combinations were commonly used in the imatinib cohort. In both cohorts, none of the patients received imatinib therapy after HSCT in their first CR. In the imatinib cohort, all patients who showed hematologic relapse after HSCT received salvage treatment comprising of imatinib and/or chemotherapy. As for the pre-imatinib cohort, 13 patients relapsed after the approval of imatinib by the Japanese government (beyond December 2001). However, we have no information on how many patients received imatinib-based therapy after their relapse. The median follow-up period for survivors was 2.6 years (range, 1.0–4.6 years) for the imatinib cohort and 6.9 years (range, 0.1–11.4 years) for the pre-imatinib cohort.

Outcome

OS and DFS. In the pre-imatinib cohort, 80 patients died after HSCT: 46 of disease recurrence and 34 of causes other than leukemia. In the imatinib cohort, 35 patients were alive, 32 of them were free of leukemia and 16 patients died after HSCT: 4 of disease recurrence and 12 of causes other than leukemia. The 3-year OS was 65% (95% confidence interval (CI), 49–78%) for the imatinib cohort and significantly higher than 44% (95% CI, 35–52%) for the pre-imatinib cohort ($P=0.0148$; Figure 1a). The 3-year DFS was 58% (95% CI, 41.8–70.9%) for the imatinib cohort and significantly higher than 37% (95% CI, 28.5–45.6%) for the pre-imatinib cohort ($P=0.039$; Figure 1b).

Table 2 shows the result of risk factor analysis for OS and DFS among all 173 patients. In the multivariate analysis, the only variable found to influence OS and DFS was the pre-transplant imatinib-based therapy (hazard ratio (HR) = 0.44 (95% CI, 0.25–0.77); $P=0.004$ and HR = 0.51 (95% CI, 0.31–0.86); $P=0.011$, respectively). The presence of cGVHD showed a tendency of favorable OS and DFS, but did not reach the statistical significance (HR = 0.66 (95% CI, 0.42–1.06); $P=0.085$ and HR = 0.75 (95% CI, 0.47–1.19); $P=0.217$, respectively).

Other outcomes of transplantation

Relapses. In the pre-imatinib cohort, 48 patients relapsed after HSCT with a median of 240 days (range, 42–2302 days).

In the imatinib cohort, 7 patients (3 of 36 with PCR negative and 4 of 12 with PCR positive at HSCT) relapsed after HSCT with a median of 137 days (range, 68–728 days). The estimated cumulative incidence of relapse at 3 years was 15.0% (95% CI, 6.6–26.7%), and significantly lower than that of the pre-imatinib cohort (50.4% at 3 years (95% CI, 39.6–60.2%); $P=0.002$; Figure 1c). Among patients in the imatinib cohort, patients with PCR negative showed significantly lower relapse rate compared with that of PCR positive (10.0% (95% CI, 2.5–23.6%) versus 41.3% (95% CI, 16.9–64.4%) at 3 years, respectively, $P=0.025$).

Non-relapse mortality. In the pre-imatinib cohort, 34 patients died of non-relapse causes at a median of 159 days (range, 5–2094 days) after HSCT. The estimated cumulative incidence of NRM in the pre-imatinib cohort was 28% (95% CI, 20–36) at 3 years (Figure 2a). In the imatinib cohort, 12 patients died of non-relapse causes at a median of 329 days (range, 41–850 days) after HSCT. The 3-year cumulative incidences of NRM were 21% (95% CI, 11–33%; Figure 2a). There were no significant differences between two cohorts ($P=0.265$).

Cause of death. Recurrence of the primary disease was the leading cause of death in both groups: 55% for the pre-imatinib cohort and 25% for the imatinib cohort. In the pre-imatinib cohort, the causes of NRM were organ failure (11%), infection (9%), GVHD (8%), transplantation-associated thrombotic microangiopathy (TMA) (4%), interstitial pneumonia (3%), graft failure (3%) and other causes (6%). In the imatinib cohort, the causes of NRM included infection (19%), bronchiolitis obliterans with organizing pneumonia (13%), TMA (13%), GVHD (13%), organ failure (6%) and other causes (12%).

Graft-versus-host disease. There was no significant difference in the cumulative incidence of Grades 2–4 aGVHD between two cohorts (31% (95% CI, 19–44%) versus 37% (95% CI, 29–46%), $P=0.391$; Figure 2b). The cumulative incidence of cGVHD at 1 year after HSCT was significantly higher in the imatinib cohort than in the pre-imatinib cohort (49% (95% CI, 31–64%) versus 27% (95% CI, 18–37%), $P=0.0261$; Figure 2c).

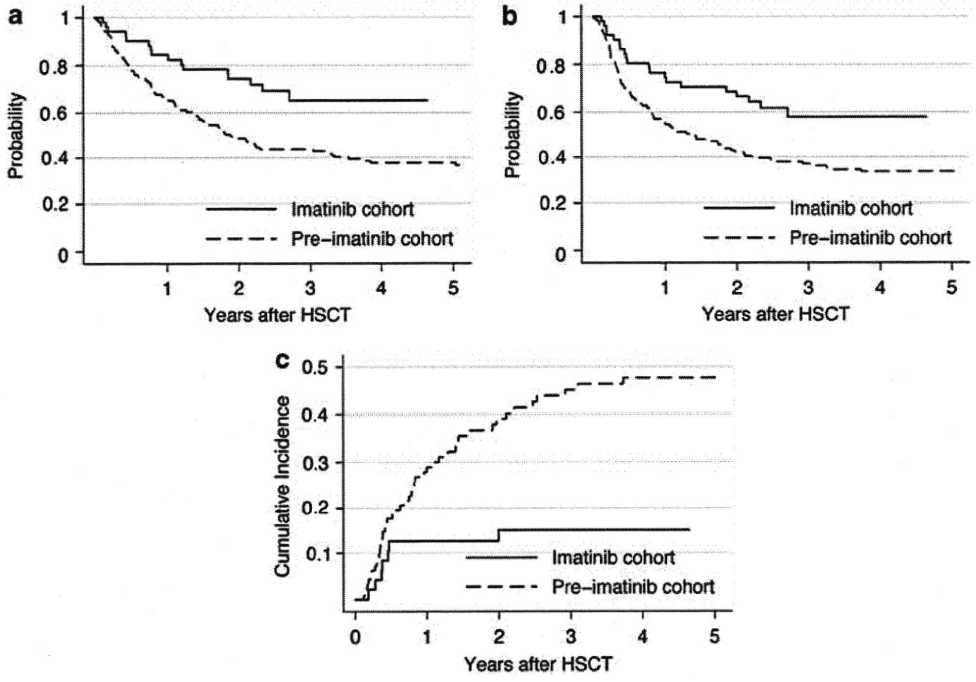

Figure 1 Transplantation outcomes of 51 patients who received imatinib-based therapy and 122 historical patients. (**a**) Overall survival, (**b**) disease-free survival and (**c**) cumulative incidence of relapse.

Table 2 Results of uni- and multivariate analysis of overall survival and disease-free survival among 173 patients with Ph+ALL

Characteristic	*Overall survival*				*Disease-free survival*			
	Univariate analysis		*Multivariate analysis*		*Univariate analysis*		*Multivariate analysis*	
	RR (95% CI)	P	*RR (95% CI)*	P	*RR (95% CI)*	P	*RR (95% CI)*	P
Imatinib-imterim therapy before HSCT	0.45 (0.26–0.77)	0.004	0.44 (0.25–0.77)	0.004	0.51 (0.31–0.83)	0.007	0.51 (0.31–0.86)	0.011
Donor status (RE versus UR)	0.87 (0.57–1.32)	0.521	0.72 (0.40–1.30)	0.275	0.77 (0.51–1.16)	0.211	0.65 (0.37–1.16)	0.147
Age at HSCT (−39 versus 40–55 versus 55−)	1.03 (0.74–1.44)	0.852	1.12 (0.78–1.62)	0.536	0.98 (0.71–1.36)	0.914	1.03 (0.73–1.47)	0.862
HLA-disparity (matched versus mismatched)	0.90 (0.39–2.06)	0.800	0.76 (0.32–1.81)	0.531	1.11 (0.49–2.54)	0.800	1.06 (0.45–2.50)	0.895
Stem-cell source (BM versus PB)	1.15 (0.72–1.82)	0.565	1.23 (0.72–2.10)	0.451	1.30 (0.85–2.00)	0.228	1.34 (0.81–2.20)	0.254
Days from diagnosis to HSCT	1.00 (0.99–1.00)	0.217	1.00 (0.99–1.00)	0.141	1.00 (0.99–1.00)	0.415	1.00 (0.99–1.00)	0.125
cGVHD as time-varying covariate (yes versus no)	0.68 (0.43–1.08)	0.101	0.66 (0.42–1.06)	0.085	0.78 (0.50–1.23)	0.292	0.75 (0.47–1.19)	0.217

Abbreviations: ALL, acute lymphoblastic leukemia; BM, bone marrow; CI, confidence interval; cGVHD, chronic graft-versus-host disease; HLA, human leukocyte antigen; HSCT, hemtopoetic stem cell transplantation; PB, peripheral blood; Ph, Philadelphia chromosome; RE, related; RR, relative risk; UR, unrelated.

However, regarding the cumulative incidence of extensive-type cGVHD, there was no significant difference between two cohorts (22% (95% CI, 10–36%) versus 12% (95% CI, 6–20%), $P=0.119$; Figure 2d).

Association between cGVHD and OS/DFS/relapse. To examine the difference of impacts of cGVHD upon clinical outcome in the pre- and imatinib cohorts, we conducted stratified analysis by cohort, treating cGVHD as a time-varying covariate (Table 3). Multivariate analysis revealed that, in the imatinib cohort, there were no significant associations between cGVHD and OS/DFS/relapse ($P=0.707$, 0.332 and 0.713, respectively). On the other hand, in the pre-imatinib cohort, there was a significant association between cGVHD and OS (HR = 0.59 (95% CI, 0.35–1.00), $P=0.048$), but not between cGVHD and DFS/relapse ($P=0.234$ and 0.338, respectively).

Engraftment. In the pre-imatinib cohort, three patients experienced graft failure. The median periods to reach the neutrophil count of $>0.5\times10^6$/l and platelet count of 50×10^6/l were 15 days (range, 8–49 days) and 25 days (range, 9–120 days), respectively, for evaluable patients. In the imatinib cohort, all 51 patients were engrafted. The median period to reach a neutrophil count of $>0.5\times10^6$/l and platelet count of 50×10^9/l was 15 days (range, 5–41 days) and 25 days (range, 11–504 days), respectively, for evaluable patients. There was no

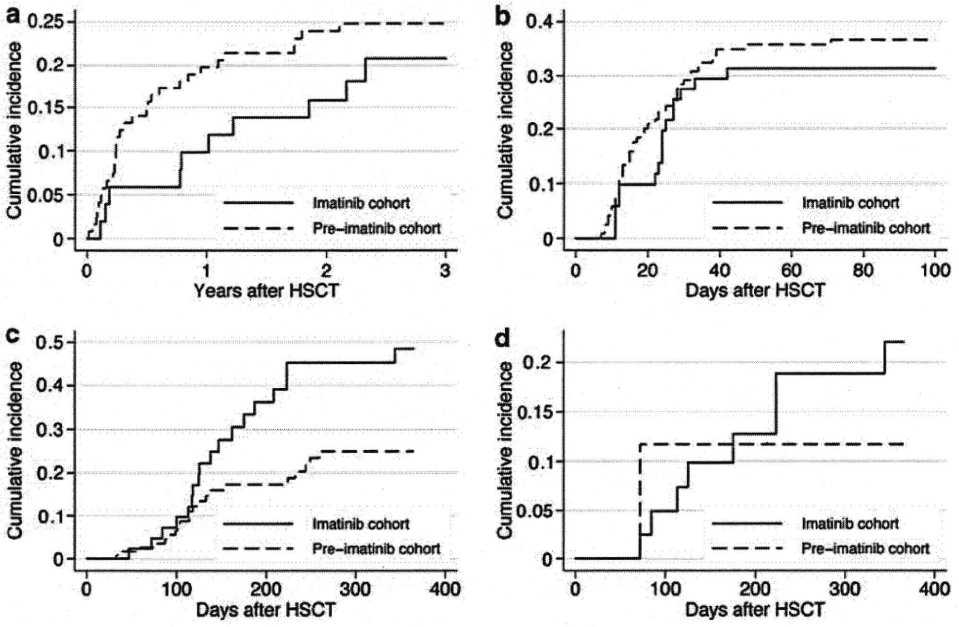

Figure 2 Cumulative incidence of GVHD or NRM. (**a**) Non-relapse mortality, (**b**) Grade 2–4 acute GVHD, (**c**) chronic GVHD and (**d**) extensive-type chronic GVHD.

Table 3 Impact of overall cGVHD on OS, DFS and relapse in multivariate analysis using cGVHD as a time-varying covariate

Cohort	*OS*			*DFS*			*Relapse*		
	Relative risk	*95% CI*	P	*Relative risk*	*95% CI*	P	*Relative risk*	*95% CI*	P
Imatinib cohort	0.80	(0.26–2.51)	0.707	0.59	(0.21–1.71)	0.332	0.74	(0.15–3.67)	0.713
Pre-imatinib cohort	0.59	(0.35–1.00)	0.048	0.73	(0.43–1.23)	0.234	0.75	(0.39–1.44)	0.388

Abbreviations: CI, confidence interval; cGVHD, chronic graft-versus-host disease; DFS, disease-free survival; HLA, human leukocyte antigen; OS, overall survival; PBSC, peripheral blood stem cell.
Data were adjusted for age categories, donors from unrelated subjects, HLA-matching status, PBSC graft and days to transplantation. Cox proportional hazard models were applied to OS and DFS, and a competing risk regression model was applied to relapse.

significant difference in neutrophil and platelet recovery between two cohorts ($P=0.201$ and 0.783, respectively).

Discussion

This study showed that patients with Ph + ALL who achieved CR by imatinib-based therapy and subsequently received allo-HSCT in their first CR showed significantly superior survival outcome to those in the pre-imatinib era. To our knowledge, our current report is the first to describe the superiority of imatinib-based therapy for this disease by analyzing a substantial number of patients with sufficient follow-up period. The treatment of Ph + ALL has changed dramatically since the introduction of imatinib and >90% of patients have achieved CR,[7,14,15] and allows SCT to be performed in a substantial proportion of patients in major or complete molecular remission.[8,16–18] Actually, in the imatinib cohort, 97 of 100 patients (97%) achieved CR and 60 (60%) could receive allo-HSCT in their first CR. Several studies reported improved OS rates compared with that in the pre-imatinib era by incorporation of imatinib-based therapy.[14,15,19,20] However, there had been few reports focusing on the clinical impact of pre-transplant imatinib administration on the outcome of HSCT. Lee *et al.*[8] reported superior outcome of HSCT by imatinib-based therapy compared with the historical control data, in which 29 patients with prior imatinib treatment showed better outcomes in terms of relapse, DFS and OS than the historical control patients. However, their comparative analysis included patients who received HSCT for refractory disease or beyond their first CR (4 of 29 patients in the imatinib group and 16 of 33 patients in the historical group). Several studies showed that remission status at the time of HSCT was one of the most important prognostic factors for outcome.[21,22] Therefore, we contend that it would be better to assess a greater number of patients and exclude patients with advanced stage at HSCT to accurately compare the clinical impact of imatinib-based therapy on the outcome of HSCT. To our knowledge, this study has the largest number of Ph + ALL patients receiving allo-HSCT in their first CR with the longest follow-up duration yet reported.

It is noteworthy from our findings that a lower rate of relapse was found in the imatinib cohort. Our results thus suggest that an imatinib-based therapy provides a survival benefit for newly diagnosed Ph + ALL patients by lowering the rate of subsequent relapse after HSCT. Despite the lack of comparative data of MRD in the pre-imatinib cohort, 75% of patients in the imatinib cohort achieved RQ–PCR negativity for *BCR/ABL* at the time of HSCT. Moreover, the relapse rate was significantly lower among

patients with PCR negative. From these, we believe that a powerful anti-leukemia activity of the imatinib-based therapy mostly contributed to the prevention of subsequent relapse after HSCT in the present analysis. Thinking of the reduced relapse rate after HSCT, impact of cGVHD should also be considered. Several studies in the pre-imatinib era reported beneficial impact of cGVHD on relapse incidence and survival.[23–25] In this study, the incidence of cGVHD was significantly higher in the imatinib cohort compared with that in the pre-imatinib cohort. In the imatinib cohort, more patients received PB as a stem cell source, which might have contributed to the high frequency of cGVHD. Besides, longer leukemia-free survival period in the imatinib cohort might have contributed to the increased frequency of cGVHD, which is a late complication often observed in the recipients of allo-HSCT who had survived without disease for at least 3 months after transplantation. One could argue that this observation could be related to a stronger graft versus leukemia effect and contribute to the lower relapse rate. However, the presence of cGVHD had no significant impact on the OS/DFS/relapse rate in our imatinib cohort by multivariate analysis.

To assist the proper interpretation of our current results, the strengths and limitations need to be considered. As discussed earlier, one of the strengths of this study is the large sample size for the imatinib cohort, which gives us a better estimation of the end points and also adds statistical power to the analyses. In addition, adjustments for potential confounders in the comparisons with the pre-imatinib cohort from a nationwide registry allow unbiased estimates to be made, at least in Japan. Given the evidence for a substantial impact of imatinib in Ph + ALL patients,[7,14–16] it is unrealistic to conduct a prospective study comparing treatments with or without imatinib. Hence, a retrospective cohort design could be suboptimal to address the key questions.

One of the possible limitations of our current analysis could be the presence of residual confounding factors both of known and unknown. Among the known factors, a difference in the conditioning regimens could be noted. The City of Hope National Medical Center reported a favorable result from the use of a fractionated TBI–etoposide regimen in the treatment of Ph + ALL.[26] However, in the comparative analysis, the clinical advantage of this approach seemed to be established mostly among patients transplanted in their second CR.[27] Moreover, this approach was commonly applied in our pre-imatinib cohort rather than in the imatinib cohort (22 and 4%, respectively). Differences in GVHD prophylaxes should also be considered. Tacrolimus was more frequently used in the imatinib cohort than in the pre-imatinib cohort, which reflects the change in practice within the field of allo-HCT in Japan as tacrolimus was widely used for unrelated allo-HSCT since 2000. Nevertheless, the lack of any differences in the incidence of aGVHD between two cohorts indicates that this factor had minimal impact in our analysis.

It may be argued that the improved outcome of the imatinib cohort have been influenced by the pre-transplant chemotherapy in the JALSG Ph + ALL 202 study. Although detailed information on the pre-transplant chemotherapy in the pre-imatinib cohort was not available, it was clear that the majority of patients were most likely treated by the JALSG ALL93 or JALSG ALL97 protocols as pre-transplant chemotherapy,[2] as these were widely used regimens in Japan at the time. The chemotherapeutic regimen in the JALSG Ph + ALL202 study was similar to those used in these protocols. Thus, the effectiveness on Ph + ALL would have been similar between the two cohorts. At least in JALSG, there had been neither remarkable progress in the chemotherapy of Ph + ALL until the clinical introduction of imatinib, nor in other groups including the MD Anderson Cancer Center.[28] Thus, in the present analysis, the influence of pre-transplant chemotherapy appears to be quite limited.

The difference of transplant year between the two cohorts (1995–2001 and 2002–2005, respectively) could have affected the outcome of HSCT, and the improvement of transplantation procedure might have contributed to the favorable outcome in the imatinib cohort. However, Nishiwaki *et al.*[29] analyzed the clinical outcome of 641 Japanese patients with Ph-negative ALL who had received allo-HSCT in their first CR in 1993–1997, 1998–2002 and 2003–2007, and reported that there was no statistical difference in OS and NRM between three periods. In this study, the incidence of NRM was lower in the imatinib cohort, but did not reach the statistical significance. Therefore, the influence of transplantation year is thought to be limited in this study.

Considering potential benefit by imatinib, the lack of information about post-transplant imatinib use in the pre-imatinib cohort might have led us to underestimate the difference between two cohorts.

In conclusion, we have found that there is a significant improvement in the OS and DFS of Ph + ALL patients who received allo-HSCT following imatinib-based therapy. Although further validation using larger cohorts from different populations is essential to confirm our findings, imatinib-based therapy is likely to be a useful strategy for not only giving patients with Ph + ALL more chance to receive allo-HSCT, but also for improving their outcome after allo-HSCT.

Conflict of interest

Dr Naoe has received research funding and honoraria from Novartis Japan. Dr Ohnishi has received research funding from Novartis Japan. Dr Miyazaki has received honoraria from Novartis Japan. The remaining authors declare no conflict of interest.

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SHORT COMMUNICATION

Eight-year follow-up of patients with immune thrombocytopenic purpura related to *H. pylori* infection

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Abstract

Thrombocytopenia related to *Helicobacter pylori* infection is a definitive subset of chronic immune thrombocytopenic purpura (ITP) but its long-term prognosis has not been evaluated extensively. The possibility of recurrence of thrombocytopenia after re-infection of *H. pylori* is another concern. We evaluated 8-year follow-up data for 11 patients with ITP related to *H. pylori* infection in a single institution. In 2001, patients with chronic ITP were evaluated for *H. pylori* infection at the Tokyo Metropolitan Komagome Hospital using ^{13}C urea breath test (UBIT). Nineteen patients turned out to be positive and were treated for *H. pylori* infection. Platelet count 6 months after treatment revealed complete response (CR) in 10 patients and response in one patient. Eight years later, 17 of these 19 patients were re-evaluated for *H. pylori* infection and platelet count. Platelet counts of the 11 previous responders remained to indicate CR. Two of 13 patients re-examined by UBIT showed positive results and one of these two patients continued to keep normal platelet count. Nonetheless, the prognosis of patients who responded to eradication was excellent.

Keywords: *ITP*, H. pylori

Introduction

The effects of eradicating *Helicobacter pylori (H. pylori)* on platelet count in patients with chronic immune thrombocytopenic purpura (ITP) were initially described in 1998, but variable results of this treatment followed [1]. Several systematic reviews have recently been performed and these variable effects have been confirmed [2, 3]. Consequently, a consensus report describing the effects of *H. pylori* on platelet count has suggested that urea breath test or stool antigen test should be considered where eradication therapy was reported to be effective [4].

Meanwhile, few reports regarding the long-term effects of eradication are available and the possibility of recurrence of thrombocytopenia after re-infection of *H. pylori* is another concern. Emilia et al., for example, reported excellent prognosis in patients who responded to initial treatment [5]. However, in this largest series reported so far, only eight responders after the eradication had been followed more than 5 years. Tsumoto et al. also recently reported the long-term efficacy of *H. pylori* eradication with excellent results in eight out of nine patients [6]. One patient relapsed but no case of re-infection was observed.

We present here 8-year follow-up data for 11 patients with ITP considered to be related to *H. pylori* infection that was successfully treated.

Materials and methods

Patients diagnosed as having ITP at the Tokyo Metropolitan Komagome Hospital and who had been involved in a study evaluating the effects of *H. pylori* infection on ITP in 2001 were included [7]. After infection was confirmed, eradication was performed and their platelet counts were followed. They were re-evaluated in 2009 for *H. pylori* infection and platelet count. Medical records were also examined for platelet counts in the interim.

Initially, ITP was defined as idiopathic thrombocytopenia (platelets less than 100×10^9/L) persisting for more than 6 months. Thrombocytopenia due to other causes, such as hepatitis C virus infection,

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drugs, lymphoproliferative disorders and other autoimmune disorders, were excluded. Normal or increased megakaryocytes in bone marrow and normal choromosomal study were also documented if available.

H. pylori infection was examined using ^{13}C urea breath test (UBIT; OTSUKA Pharm. Co., Ltd, Tokyo, Japan). Eradication of *H. pylori* was performed using standard therapy: amoxicillin (1000 mg, twice daily), clarithromycin (250 mg, three times daily) and proton-pump inhibitor (20–40 mg, twice daily) for 1 week.

The results of treatment were evaluated by platelet count 6 months after the eradication without UBIT. Complete response (CR) was defined as a platelet count more than 100×10^9/L and absence of bleeding. Response (R) was defined as a platelet count of more than 30×10^9/L, a minimum two-fold increase in baseline platelet count and absence of bleeding. No response (NR) was a platelet count of less than 30×10^9/L, a less than two-fold increase in baseline platelet count or bleeding. Loss of CR or R was defined as a platelet count below 100×10^9/L or bleeding (from CR), or a platelet count below 30×10^9/L, a less than two-fold increase in baseline platelet count or bleeding (from R) [8]. After 8 years, patients were re-evaluated by UBIT.

This study was approved by Ethics committee and consented by the participants.

Results

In 2001, 31 patients with chronic ITP were evaluated for *H. pylori* infection. Nineteen of these were found to be positive and were treated for *H. pylori* infection. The rest of them had never revealed normal platelet count during their follow-up periods except for one after splenectomy and another with steroid treatment. Eight years later, 13 of these 19 patients were re-evaluated for *H. pylori* infection and platelet count was followed in 17. Two patients were lost at 5 years after treatment.

At the time of eradication treatment, mean age was 59.7 years (29–74 years), eight patients were male and median length from diagnosis was 9.3 years (0–28 years). Nine patients had received steroid treatment and splenectomy had been performed in three (Table I).

Initially, eight out of 19 patients did not respond to treatment and they remained NR after 8 years. One of these was re-diagnosed as myelodysplastic syndrome and received hematopoietic stem cell transplantation while another was diagnosed as having liver cirrhosis and related thrombocytopenia. Before eradication, platelet count exceeded 100×10^9/L in two patients, partly because of steroid treatment, and their platelet counts remained below the normal range after eradication.

The platelet counts of the remaining 11 patients were initially below 50×10^9/L. Within 6 months after treatment for *H. pylori,* 10 patients showed CR and the remainder showed R. These responses had been sustained for 8 years except for one patient, who was lost follow-up after 5 years and by that time normal platelet count had been documented. Steroid treatment was discontinued in all three patients and no relapse was observed or reported.

Two of the 13 patients examined for *H. pylori* infection using UBIT after 8 years turned out to be positive, but the timing of infection was unknown. Even though UBIT test suggested re-infection of *H. pylori,* platelet count of the first patient had been in normal range (UPN 9). The second patient did not respond to eradication and remained positive for *H. pylori* infection 6 months after treatment, but responded to the same eradication treatment in 2009 and became CR (UPN 6).

Discussion

Thrombocytopenia related to *H. pylori* infection is a definitive group of ITP and its prognosis is excellent if patients respond to treatment. Although the predictors of platelet response in patients who are found to be *H. pylori* positive are not known, the responses are usually observed within 6 months after eradication [7]. However, there are few reliable data regarding the long-term effects of treatment available. The possible recurrence of thrombocytopenia after re-infection of *H. pylori* is another concern. Several reports revealed excellent prognosis of the patients who responded to eradication but sample size is small, length of follow-up is rather short and the possibility of re-infection was not fully evaluated [5, 6]. Because of the shortage of evaluable patients, it would be worth accumulating the data obtained from available patients to draw any confirmative conclusions.

In this regard, this paper is also dealing with a small number of patients who responded to treatment. They remained free from thrombocytopenia and no relapse has been observed during the last 8 years. In addition, one patient showed possible re-infection after resolving thrombocytopenia but the platelet count remained in CR. This observation may suggest the possibility that re-infection may not necessarily induce thrombocytopenia, but further studies are needed.

The incidence of re-infection of *H. pylori* is another factor to be considered when the prognosis of the patients with ITP related to *H. pylori* was concerned. According to Archimandritis et al., the