Impact of hematogones on the long-term outcomes of single-unit cord blood transplantation for adult patients

Running title: Hematogones on outcomes of CBT

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Abstract

Hematogones are normal B-lymphocyte precursors identified in the regenerative state of the bone marrow following allogeneic hematopoietic stem cell transplantation (HSCT). To evaluate the impact of hematogones on long-term outcomes after single-unit cord blood transplantation (CBT), we retrospectively analyzed 134 adult patients at our institute. At the median time of 41 days (range, 20 to 77 days) after CBT, the median proportion of morphological hematogones in bone marrow was found to be 2.4% (range, 0 to 13.0%). In the patients with standard-risk, the higher proportion of morphological hematogones was associated with lower transplant-related mortality after CBT. The proportion of hematogones did not affect the subsequent absolute lymphocyte counts in the peripheral blood and serum immunoglobulin G levels 6 months later after CBT. These data shows that morphological hematogones in the routine bone marrow analysis might be a practical and easily evaluable method of predicting outcomes after CBT.

Introduction

Cord blood transplantation (CBT) is an acceptable alternative to the unrelated allogeneic hematopoietic stem cell transplantation (HSCT) for the adult patients without human leukocyte antigen (HLA)–matched related or unrelated donors.[1,2] The limited cell dose in a single cord blood unit remains the disadvantage of CBT, which might contribute to a higher incidence of graft failure, delayed hematopoietic recovery, and poor immune reconstitution, leading to higher transplant-related mortality (TRM) or overall mortality after CBT.[2-4]

Hematogones, that are normal B-lymphocyte precursors, are identified in the bone marrow of healthy children, and might be increased in various diseases and regenerative conditions following chemotherapy and HSCT.[5-11] Recently, several studies have demonstrated that a higher percentage of hematogones was associated with better clinical outcomes after conventional chemotherapy[12-14] or allogeneic HSCT.[15-18] However, investigation of the impact of hematogones on outcomes after CBT has been limited.[11,15,16] Therefore, we retrospectively examined the impact of hematogones on long-term outcomes after single-unit CBT for the adult patients.

Patients and methods

Patients and transplantation procedures

Between February 2003 and May 2015, we conducted a single-unit CBT as first allogeneic HSCT for 183 adult patients at the Institute of Medical Science, The University of Tokyo. To evaluate the impact of hematogones on long-term outcomes after CBT at the first bone marrow analysis within 90 days, we excluded 16 patients who relapsed or died within 90 days, 11 patients who were not evaluated for bone marrow analysis and/or chimerism

status within 90 days, and 22 patients whose chimerism were not complete donor chimerism that was defined by the presence of more than 95% donor-derived cells, at first bone marrow analysis after CBT. Finally, the remaining 134 patients who survived more than 90 days without relapse were analyzed in this study. Almost all patients received the same supportive care, such as antibacterial, antifungal, and antiviral agents, as previously described.[2] To reduce the duration of neutropenia after CBT, all patients received granulocyte colony-stimulating factor (G-CSF) starting from day one until the achievement of durable neutrophil engraftment. If the serum immunoglobulin G (IgG) level was lower than 400 mg/dl with or without active infectious complications, intravenous immunoglobulin was used until three months after CBT. This retrospective study was approved by the institutional review board of the Institute of Medical Science, The University of Tokyo.

Quantitative analysis of Hematogones

The quantitative analysis of hematogones was performed at the first bone marrow analysis after CBT. The bone marrow analysis was routinely performed to evaluate the residual disease and chimerism status related to the discontinuation of the G-CSF, as the effect of granulocytes on G-CSF administration could be minimized. The bone marrow aspirate smears were stained with May-Giemsa and reviewed to focus on the proportion of hematogones present in the total nucleated cells of the bone marrow. We defined hematogones as the lymphoid progenitor cells with the diameter varying from 10 to 20 μm, scanty cytoplasm, and condensed homogenous nuclear chromatin. We evaluated 500 cells in each smear, with a minimum of 300 cells being evaluated in the smears with low cellularity. In 25 of 134 samples, the proportion of morphological hematogones was evaluated by the two reviewers. Because the correlation between the proportions of

morphological hematogones determined by the two reviewers was found to be significant ($r=0.85$, $P<0.001$), the remaining samples were jointly reviewed. Flow cytometry of bone marrow samples was routinely performed for detection of residual disease in a commercial laboratory (BML, INC, Tokyo, Japan).

Endpoints and definitions

The primary endpoint of the study was TRM at five years. Secondary endpoints were presented by overall survival (OS) and relapse rate at five years. To evaluate the impact of hematogones within 90 days after CBT on OS, relapse, and TRM, we performed landmark analysis.[19] Therefore, the incidences of OS, relapse, and TRM were evaluated in the patients surviving for more than 90 days without relapse. OS (inverse of overall mortality) was defined as the time from the date of CBT to the date of death or last observation. Relapse was defined by the hematological evidence of disease. TRM was defined as death during a remission. The disease status at CBT was classified as standard risk or high risk. Complete remission (CR) 1 or CR2 without poor prognostic karyotype for acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), refractory anemia with myelodysplastic syndrome (MDS), chronic phase for chronic myelogenous leukemia (CML), CR1 or CR2 for lymphoma, and severe aplastic anemia (SAA) were classified as standard risk, whereas patients in all other situations were classified to be at high risk, as previously described.[1,20] The number of HLA disparities between recipient and cord blood was defined as low resolution for HLA-A and -B and high resolution for HLA-DRB1. The cord blood units were obtained from the Japan Cord Blood Bank Network.

Statistical analysis

The categorical variables were compared using the chi-square test. The continuous variables were compared with the Mann-Whitney U test. The Spearman rank correlation coefficient was calculated to assess the correlation between the morphological and flow cytometric hematogones or patients' age. The probability of OS was estimated according to the Kaplan–Meier method and the groups were compared using the log-rank test. The estimation of probabilities of the relapse and TRM were based on a cumulative incidence method to accommodate competing risks, and the groups were compared using Gray's test. Multivariate analysis was performed with a Cox proportional hazard model for overall mortality, and a Fine and Gray proportional hazards model for relapse and TRM using the following factors: age (≥45 vs. <45 years), disease status at CBT (high risk vs. standard risk), cord blood nucleated cell count (≥2.5×10^7/kg vs. <2.5×10^7/kg), cord blood CD34+ cell count (≥1×10^5/kg vs. <1×10^5/kg), HLA disparities (≥3 vs. <3), and the proportion of hematogone cells (≥1% vs. <1%), as shown below. All the statistical analyzes were performed by using the EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).[21] P values of <0.05 were considered to be statistically significant.

Results

Characteristics of patients and transplantation outcomes

The characteristics of patients, cord blood units, and transplantation procedures are shown in Table 1. The median age was 42 years (range, 16 to 64 years). The disease types included AML (n=70), ALL (n=33), MDS (n=16), CML (n=6), lymphoma (n=7), multiple myeloma (n=1), and SAA (n=1). There were 71 high-risk patients at CBT (53%).

The most common conditioning regimen was total body irradiation (TBI)+Cyclophosphamide (CY)+cytosine arabinoside (Ara-C)/G-CSF(60%) for myeloid malignancies, and TBI+CY+Ara-C (23%) for lymphoid malignancies, as previously described.[22,23] No patients received anti-thymocyte globulin (ATG) containing conditioning regimen. The majority of graft-versus-host disease (GVHD) prophylaxis was cyclosporine + methotrexate (96%). No patients received corticosteroids for prophylaxis of GVHD. The median number of cryopreserved nucleated cells was 2.50×10^7/kg (range, 1.51 to 5.69×10^7/kg), and a median number of cryopreserved CD34+ cells was 0.92×10^5/kg (range, 0.27 to 2.81×10^5/kg). The median follow-up period for survivors after CBT was 78 months (range, 3-153 months).

Characteristics of hematogones after CBT

At the median time of the first bone marrow analysis at 41 days (range, 20 to 77 days) after CBT, the median proportion of morphological hematogones in the total nucleated cells of the bone marrow was found to be 2.4% (range, 0 to 13.0%). The median proportions of $CD45^{dim}$ SSC^{low} CD10+ cells and $CD45^{dim}$ SSC^{low} CD19+ cells by flow cytometry were 4.14% (range, 0-30.19) and 2.07% (range, 0-21.86), respectively (Table1). The proportion of morphological hematogones was significantly correlated with the proportion of $CD45^{dim}$ SSC^{low} CD10+cells ($r=0.7$, $P<0.001$, Figure1A) and $CD45^{dim}$ SSC^{low} CD19+cells ($r=0.7$, $P<0.001$, Figure1B) by flow cytometry, respectively. The correlation between the proportion of morphological hematogones and patients' age was found to be modest but significant ($r=-0.20$, $P=0.01$) (Figure 1C).

To evaluate the impact of hematogones on outcomes after CBT, patients were grouped according to the proportion of hematogones and divided into four categories (<1%, 1-2%, 2-3%, ≥3%). The patients who had <1% hematogones showed inferior OS

and TRM compared to other three groups, although this was not statistically significant (data not shown). Based on these findings, the cutoff point of the proportion of hematogones was 1% in this study. There were no significant differences in the characteristics of the patients who had <1% as well as ≥1% hematogones, except for the cases based on patients' age, conditioning regimen, and GVHD prophylaxis (Table 1).

Prognostic impact of hematogones on long-term outcomes following CBT

The rates of OS, relapse, and TRM did not differ significantly between the patients with <1% hematogones and those with ≥1% hematogones in a univariate analysis (Figure 2A-C). In multivariate analysis, the hazard risks of overall mortality, relapse, and TRM were also not significantly different in the group of patients with <1% and ≥1% hematogones (Table 2).

We also analyzed a subgroup of patients with standard-risk or high-risk of disease status followed by CBT. We were unable to find any impact of hematogones on the survival rate relating to disease risk at CBT in univariate (Figure 3A, B) and multivariate analysis (Table 2). The proportion of hematogones was not associated with a cumulative incidence of relapse (Table 2). Among standard-risk patients, the cumulative incidence of TRM was significantly lower in patients with ≥1% hematogones compared to those with <1% hematogones in univariate (P=0.04 by Gray test) (Figure 3C) and multivariate analysis (hazard risk 0.23, P=0.04) (Table 2). In contrast, there was no significant difference observed among the high-risk patients in the cumulative incidence of TRM between the two groups in univariate (Figure 3D) and multivariate analysis (Table 2). We also analyzed a subgroup of patients with myeloid [AML, MDS, CML] or lymphoid [ALL, NHL, MM] disease, but we were unable to find any impact of hematogones on outcomes after CBT for each disease type (data not shown).

Effect of hematogones on immune reconstitution after CBT

We analyzed whether the proportion of hematogones could affect the subsequent recovery of absolute lymphocyte counts in the peripheral blood and serum IgG levels at 3, 6, 9, and 12 months after CBT. At three months after CBT, the absolute lymphocyte counts were significantly higher in patients with ≥1% hematogones compared to those with <1% hematogones. However, there were no significant differences in the absolute lymphocyte counts between the two groups at 6, 9, and 12 months after CBT (Figure 4A). No significant differences were observed in serum IgG levels between the two groups at 3, 6, 9, and 12 months after CBT (Figure 4B).

Discussion

The purpose of this retrospective study was to evaluate the impact of morphological hematogones in the first bone marrow analysis on long-term outcomes after CBT for adult patients. Since it was difficult to distinguish hematogones from neoplastic lymphoblast by morphological and flow cytometric analysis, the patients demonstrating complete donor chimerism in the first bone marrow analysis and surviving more than 90 days without relapse were included in this study. Moreover, we evaluated the proportion of hematogones by morphological bone marrow smears in clinical practice, as flow cytometry was routinely performed only for the detection of residual diseases. Although optimal methods for evaluation of hematogones have yet to be clarified, there was a significant correlation between the morphological and flow cytometric hematogones observed in the present study. However, manipulations of the flow cytometric analysis, such as washing, centrifugation, and red blood cell lysis, could result in loss of erythroblast and granulocyte

cells, and different combination of surface markers has been used for detection of hematogones by flow cytometric analysis.[11,16-18] Furthermore, the proportion of hematogones has been reported to be higher after CBT than BMT,[8,16] which is partly due to the higher generative capacity of B-lymphocyte progenitors from cord blood as compared to the adult bone marrow cells.[24] These data indicated that meaningful clinical threshold for hematogones could be dependent on a method to evaluate hematogones, type of stem cell source, and the proportion of hematogones made from total nucleated cell counts or mononuclear cell counts.

Previous studies have reported the associations between the higher proportion of hematogones and better survival rate after allogeneic HSCT.[15-18] In the CBT setting, there were three studies reporting the associations between the hematogones and post-transplantation outcomes. Although Montesinos et al. reported no impact of morphological hematogones at a median of 101 days on the survival and relapse rate in 165 CBT recipients,[11] Honebrink et al. reported that a higher proportion of morphological hematogones at day 100 was associated with better survival rate, due to lower TRM in single or double CBT for 88 AML patients.[15] Shima et al. also reported better survival results with the higher proportion of hematogones evaluated by flow cytometry at the time of engraftment in 49 CBT patients.[16] Regardless of the threshold level and method of evaluation of hematogones, the improvement in the survival rate might be due to the reduction of TRM and not the relapse incidence.[15,18] Moreover, several studies showed the association of higher proportion of hematogones with lower incidences of severe acute GVHD[15,16,18] and extensive chronic GVHD[11], which might contribute to the lower TRM after allogeneic HSCT. In our study, a higher proportion of morphological hematogones at a median of 41 days was associated with lower TRM after single-unit CBT in patients with standard-risk. Since the timing of evaluation for hematogones was quite different: before, during, or after acute GVHD, the effect of hematogones on acute GVHD might be difficult

to evaluate in our study. On the other hand, the proportion of hematogones was not associated with the cumulative incidence of extensive chronic GVHD in a univariate and multivariate analysis (data not shown). The causes of TRM in our cohort were GVHD in 6 patients, post-transplant lymph proliferative disorder in 2, invasive aspergillosis in 1, heart failure in 1, intestinal pneumonia in 1, and secondary cancer in 1 patient. Since the number of patients who experienced TRM 90 days after CBT was too small in the present study, we were unable to clarify the cause of TRM according to the proportion of hematogones. However, similar to the previous studies,[15,18] our data suggested that proportion of hematogones was a useful predictor of TRM after CBT at least in the patients with standard-risk.

CBT has been associated with delayed reconstitution of numbers and function of T-lymphocyte[3,4,25-28] that contribute to an increased incidence of opportunistic viral infection.[29] By contrast, B-lymphocyte reconstitution was faster in CBT than the bone marrow transplantation.[27,28] Nakatani et al. demonstrated that faster B-lymphocyte reconstitution in CBT compared to allogeneic HSCT from other adult sources was not due to homeostatic expansion but B cell neogenesis using the measurement of kappa-deleting recombination excision circles (KRECs) level.[30] Therefore, the higher proportion of hematogones observed in the bone marrow might have been associated with B-lymphocyte reconstitution in peripheral blood, which might contribute to the reconstitution of humoral immunity after CBT. In fact, a higher proportion of hematogones has been reported to be associated with reconstitution of B-lymphocyte counts in the peripheral blood[16,17] and serum IgG levels[18] after allogeneic HSCT, whereas another study has reported no association between the proportion of hematogones and recovery of absolute lymphocyte counts after CBT.[15] Our data showed that the proportion of hematogones did not affect the subsequent recovery of absolute lymphocyte counts in the peripheral blood and serum IgG levels six months later after CBT. Immune reconstitution

could be affected by several factors, such as conditioning regimen including ATG, prophylaxis and treatment of GVHD, patients' age, stem cell source, and HLA disparity. Our study included a relatively homogeneous adult patient population treated with single-unit CBT following the conditioning regimens without ATG and a cyclosporine-based GVHD prophylaxis without corticosteroids. Since the administration of systemic corticosteroids for treatment of GVHD may affect the proportion of hematogones, we analyzed the impact of hematogones on OS and TRM in the patients who did not receive prednisolone at ≥ 0.5 mg/kg for more than seven days before evaluation of hematogones (n=121). However, similar results were observed only for patients who did not receive any corticosteroids for the treatment of GVHD (data not shown).

One limitation of this study is the different timing of evaluation for hematogones. Several studies have shown that the proportion of hematogones depended on the time after allogeneic HSCT,[15,17] whereas another study did not.[16] In addition, the timing of hematogone quantitation was different from each study.[15-18] Although the median day of evaluation for hematogones after CBT did not differ significantly between the patients with <1% hematogones and those with ≥1% hematogones in our study (P=0.06), the difference timing of hematogone quantitation may affect the results of this study.

In conclusion, morphological hematogones in routine bone marrow analysis is a practical and easily evaluable method of predicting outcomes after CBT. Our data showed that the proportion of hematogones affected TRM in the standard-risk patients. Further studies are warranted to evaluate the impact of hematogones on the outcomes after single-unit CBT.

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Potential conflict of interests

The authors declare no competing financial interests.

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Figure legends

Figure 1. Correlation between the proportion of morphological and flow cytometric hematogones or patients' age.

(A) The relationship between the proportion of morphological hematogones and $CD45^{dim}$ SSC^{low} CD10+ cells by flow cytometry*(r=0.7, P<0.001 by Spearman rank correlation test).

(B) The relationship between the proportion of morphological hematogones and $CD45^{dim}SSC^{low}$CD19+cells by flow cytometry* (r=0.7, P<0.001 by Spearman rank correlation test).

(C) The relationship between the proportion of morphological hematogones and patients' age (r=-0.20, P=0.01 by Spearman rank correlation test).

* Available data of flow cytometry was obtained from 127 patients.

Figure 2. The probability of overall survival (OS) and cumulative incidences of relapse and transplant-related mortality (TRM) after cord blood transplantation (CBT) according to the proportion of hematogones.

(A) The probabilities of OS at five years were 69.7% (95% confidence interval [CI], 48.1 to 83.7%) for patients with <1% hematogones and 80.9% (95% CI, 70.9 to 87.7%) for patients with ≥1% hematogones (P=0.45 by log-rank test).

(B) The cumulative incidence of relapse at five years was 14.9% (95% CI, 5.3 to 29.0%) for patients with <1% hematogones and 19.6% (95% CI, 12.2 to 28.4%) for patients

with ≥1% hematogones (P=0.84 by Gray test).

(C) The cumulative incidence of TRM at five years was 19.2% (95% CI, 6.6 to 36.6%) for patients with <1% hematogones and 5.8% (95% CI, 2.1 to 12.3%) for patients with ≥1% hematogones (P=0.16 by Gray test).

Figure 3. The probability of overall survival (OS) and cumulative incidences of transplant-related mortality (TRM) after cord blood transplantation (CBT) according to the proportion of hematogones among the patients with standard-risk or high-risk.

(A) The probabilities of OS at five years were 68.9% (95% CI, 36.3 to 87.2%) for patients with <1% hematogones and 83.6% (95% CI, 68.5 to 91.9%) for patients with ≥1% hematogones among the standard-risk patients (P=0.18 by log-rank test).

(B) The probabilities of OS at five years were 70.1% (95% CI, 38.5 to 87.6%) for patients with <1% hematogones and 78.3% (95% CI, 63.3 to 87.7%) for patients with ≥1% hematogones among the high-risk patients (P=0.85 by log-rank test).

(C) The cumulative incidence of TRM at five years was 24.4% (95% CI, 5.1 to 51.3%) for patients with <1% hematogones and 4.9% (95% CI, 0.8 to 15.1%) for patients with ≥1% hematogones among the standard-risk patients (P=0.04 by Gray test).

(D) The cumulative incidence of TRM at five years was 14.8% (95% CI, 2.1 to 38.7%) for patients with <1% hematogones and 6.7% (95% CI, 1.7 to 16.8%) for patients with ≥1% hematogones among the high-risk patients (P=0.94 by Gray test).

Figure 4. The recovery of absolute lymphocyte counts in peripheral blood (PB) and serum IgG levels after cord blood transplantation (CBT) according to the proportion of

hematogones.*

(A) The recovery of absolute lymphocyte counts in PB at 3, 6, 9, and 12 months after CBT.†

(B) The recovery of serum IgG levels at 3, 6, 9, and 12 months after CBT.‡

* Data are shown as the mean ± standard deviation.

† Available data of absolute lymphocyte counts in PB was obtained from 133, 124, 111, and 105 patients at 3, 6, 9, and 12 months after CBT, respectively.

‡ Available data of serum IgG levels was obtained from 133, 116, 103, and 97 patients at 3, 6, 9, and 12 months after CBT, respectively.

Table 1. Characteristics of the patients, grafts, and transplantation

Characteristics	Entire cohort	Hematogones <1%	Hematogones ≥1%	P-value
Number of patients	134	35	99	
Sex, number (%)				1.00
Male	79 (59%)	21 (60%)	58 (59%)	
Female	55 (41%)	14 (40%)	41 (41%)	
Age, years, median (range)	42 (16-64)	46 (23-64)	41 (16-62)	0.008
CMV serostatus, number (%)				0.06
Positive	118 (88%)	34 (97%)	84 (85%)	
Negative	16 (12%)	1 (2%)	15 (15%)	
Disease type, number (%)				0.28
AML	70 (52%)	17 (49%)	53 (53%)	
ALL	33 (25%)	8 (23%)	25 (25%)	
MDS	16 (12%)	3 (9%)	13 (13%)	
CML	6 (5%)	2 (6%)	4 (4%)	
NHL	7 (5%)	4 (11%)	3 (3%)	
MM	1 (<1%)	1 (3%)	0	
SAA	1 (<1%)	0	1 (1%)	
Disease risk at CBT, number (%)				0.69
Standard-risk	63 (47%)	15 (43%)	48 (49%)	
High-risk	71 (53%)	20 (57%)	51 (52%)	
Conditioning regimen				0.03
TBI12y+CY+Ara-C/G-CSF	81 (60%)	17 (49%)	64 (65%)	
TBI12y+CY+Ara-C	31 (23%)	9 (26%)	22 (22%)	
TBI12Gy+others	15 (11%)	4 (11%)	11 (11%)	
TBI<12Gy+others	7 (5%)	5 (14%)	2 (2%)	
GVHD prophylaxis, number (%)				0.005
CSP+MTX	128 (96%)	30 (86%)	98 (99%)	
CSP+MMF	6 (5%)	5 (14%)	1 (1%)	
Number of nucleated cells, $\times 10^7$/kg, median (range)	2.50 (1.51-5.69)	2.52 (1.73-3.97)	2.49 (1.51-5.69)	0.63
Number of $CD34^+$cells, $\times 10^5$/kg, median (range)	0.92 (0.27-2.81)	0.93 (0.32-2.81)	0.92 (0.27-2.64)	0.54

HLA disparities, number (%)				0.35
1	12 (9%)	5 (14%)	7 (7%)	
2	73 (55%)	20 (57%)	53 (53%)	
3	46 (34%)	9 (26%)	37 (37%)	
4	3 (2%)	1 (3%)	2 (2%)	
ABO incompatibility, number (%)				0.48
Match	37 (28%)	10 (29%)	27 (27%)	
Major mismatch	36 (27%)	12 (34%)	24 (24%)	
Minor mismatch	40 (30%)	10 (29%)	30 (30%)	
Bidirectional mismatch	21 (16%)	3 (9%)	18 (18%)	
Sex compatibility, number (%)				0.83
Female donor to male recipient	42 (31%)	10 (29%)	32 (32%)	
Other	92 (69%)	25 (71%)	67 (68%)	
Follow-up for survivors, months, median (range)	78 (3-153)	105 (3-153)	78 (5-150)	0.97
Days of evaluation for hematogones after CBT, median (range)	41 (20-77)	39 (21-74)	42 (22-77)	0.06
The proportion of $CD45^{dim}$ SSC^{low} CD10+ cells by flow cytometry, median (range)	4.14 (0-30.19)	0.59 (0-7.60)	5.60 (0.04-30.19)	<0.001
The proportion of $CD45^{dim}$ SSC^{low} CD19+ cells by flow cytometry, median (range)	2.07 (0-21.86)	0.21 (0-5.83)	3.22 (0.01-21.87)	<0.001

Note: cytomegalovirus, CMV; Acute myeloid leukemia, AML; Acute lymphoblastic leukemia, ALL; Myelodysplastic syndromes, MDS; Chronic myelogenous leukemia, CML; Non-Hodgkin's lymphoma, NHL; Multiple myeloma, MM; Severe aplastic anemia, SAA; Cord blood transplantation, CBT; total boby irradiation, TBI; cyclophosphamide, CY; cytosine arabinoside, Ara-C; granulocyte colony-stimulating factor, G-CSF; Graft versus host disease, GVHD; cyclosporine A, CSP; Methotrexate, MTX; mycophenolate mofetil, MMF; human leukocyte antigen, HLA.

Table 2. Multivariate analysis for overall mortality, relapse, and transplant-related mortality (TRM)

	Overall mortality		**Relapse**		**TRM**	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Entire cohort						
Hematogones<1%	1		1		1	
Hematogones≥1%	0.72 (0.31-1.64)	0.43	1.34 (0.51-3.53)	0.55	0.43 (0.07-2.35)	0.33
Standard-risk						
Hematogones<1%	1		1		1	
Hematogones≥1%	0.87 (0.25-2.92)	0.82	1.31 (0.26-6.43)	0.74	0.23 (0.05-0.95)	0.04
High-risk						
Hematogones<1%	1		1		1	
Hematogones≥1%	0.63 (0.20-2.02)	0.44	1.26 (0.36-4.43)	0.71	0.64 (0.09-4.41)	0.65

Note: Hazard rario, HR; Confidence interval, CI.

Figure 1. Ishii H et al

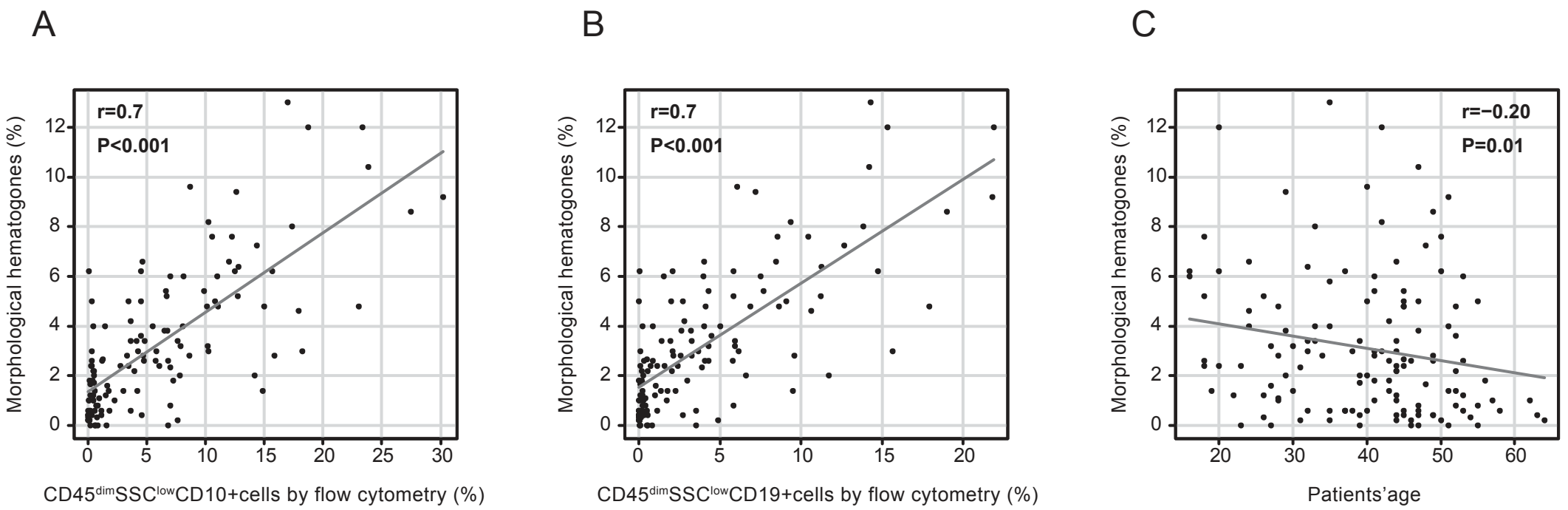

Figure 2. Ishii H et al

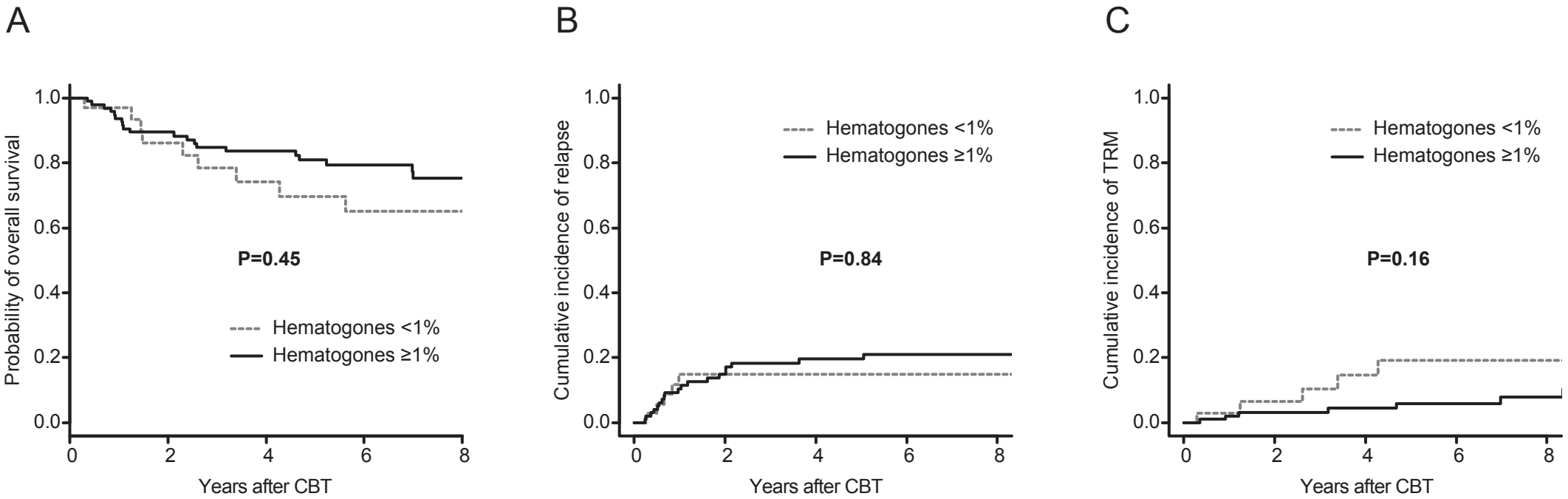

Figure 3. Ishii H et al

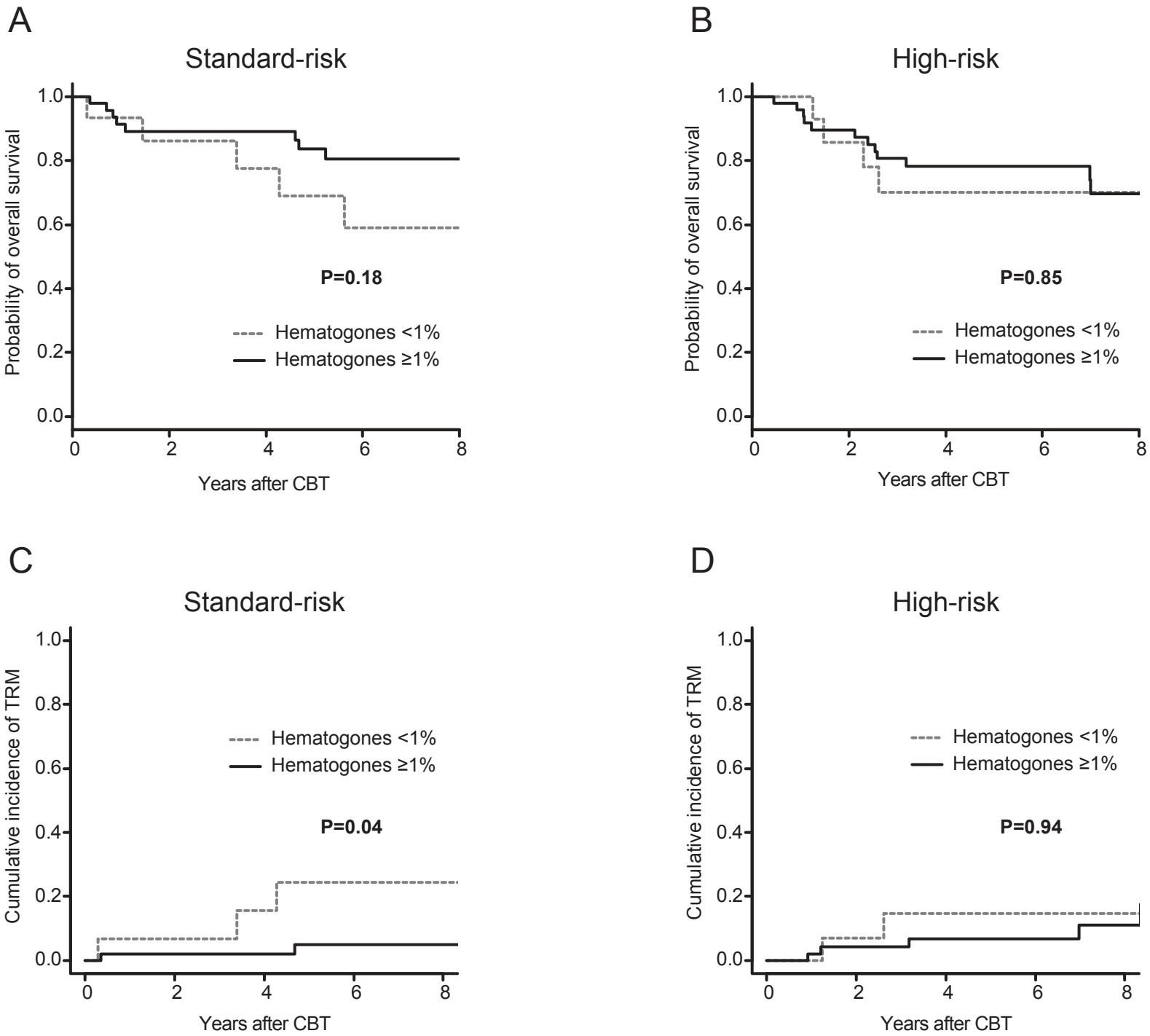

Figure 4. Ishii H et al