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Address for Correspondence:
Keon Hee Yoo, MD, PhD
Department of Pediatrics, Samsung Medical
Center, Sungkyunkwan University School of
Medicine, 81 Irwon-ro, Gangnam-gu,
Seoul 06351, Korea.
E-mail: hema2170@skku.edu

ORCID iDs
Young Bae Choi
https://orcid.org/0000-0001-7016-8827
Ji Won Lee
https://orcid.org/0000-0003-0084-1304
Ki Woong Sung
https://orcid.org/0000-0001-5989-4772
Hong Hoe Koo
https://orcid.org/0000-0001-8082-1412
Hee-Jin Kim
https://orcid.org/0000-0003-3741-4613
Keon Hee Yoo
https://orcid.org/0000-0002-5980-7912

Impact of Day 14 Peripheral Blood Chimerism after Allogeneic Hematopoietic Stem Cell Bone Transplantation on the Treatment Outcome of Non-Malignant Disease

Young Bae Choi [ID],[1] **Ji Won Lee** [ID],[2] **Ki Woong Sung** [ID],[2] **Hong Hoe Koo** [ID],[2] **Hee-Jin Kim** [ID],[3] and **Keon Hee Yoo** [ID][2,4,5]

[1]Department of Pediatrics, Chungbuk National University Hospital, Cheongju, Korea
[2]Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea
[3]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea
[4]Department of Health Science and Technology, SAIHST, Sungkyunkwan University, Seoul, Korea
[5]Stem Cell & Regenerative Medicine Institute, Samsung Medical Center, Seoul, Korea

ABSTRACT

Background: The impact of early peripheral blood chimerism on the outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT) is unclear. We aimed to determine whether day 14 peripheral blood chimerism after allo-HSCT predicts outcomes in patients with non-malignant diseases.

Methods: Data from 56 patients who received allo-HSCT between April 2007 and March 2016 were retrospectively analyzed. Chimerism was evaluated using short-tandem repeat polymerase chain reaction, with mixed chimerism (MC) defined as greater than 1% recipient cells which was further categorized into low-level MC (> 1% and < 15% of recipient-derived cells) and high-level MC (≥ 15% of the recipient-derived cells).

Results: Thirty-six patients showed complete donor chimerism (CC), 14 low-level MC, and 6 high-level MC at day 14 post-transplant. The estimated 5-year event-free survival (EFS) was higher in the CC or low-level MC groups than in the high-level MC group (86.1% vs. 71.4% vs. 33.3%; $P = 0.001$). In BM or peripheral blood stem cell (BM/PBSC) transplants, the 5-year EFS was higher in the CC or low-level MC group than in the high-level MC group (93.1% vs. 66.7% vs. 0%; $P < 0.001$). However, in cord blood transplants, the 5-year OS and EFS according to the day 14 peripheral blood chimerism did not reach statistical significance.

Conclusion: Although CC is not always necessary after allo-HSCT for non-malignant diseases, our data suggest that day 14 peripheral blood chimerism may predict outcomes in patients with non-malignant diseases who underwent BM/PBSC transplants.

Keywords: Chimerism; Allogeneic Hematopoietic Stem Cell Transplantation; Non-Malignant Disease

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been a curative modality for various non-malignant diseases, including bone marrow failure syndromes (BMFS), primary immunodeficiency diseases, hemophagocytic lymphohistiocytosis, inherited metabolic disorders, and many other hematologic disorders.[1-4] Analysis of the chimerism status has become an important diagnostic tool for post-transplant investigation of engraftment and prediction of disease recurrence. Previous reports showed that a malignant disease with increasing mixed chimerism (MC) after allo-HSCT had the highest risk of relapse, and an early intervention could improve the transplant outcomes.[5,6] In contrast to malignant diseases, stable MC even with a high degree of residual recipient hematopoiesis, allows for the correction of the underlying non-malignant diseases.[7,8] However, because MC may progress to graft rejection, early detection of the chimerism status after allo-HSCT might be important to prevent graft loss in non-malignant diseases. Moreover, early analysis of the chimerism status could provide information for subsequent interventions, such as rapid withdrawal of immunosuppression, donor lymphocyte infusion, or stem cell boost.[9]

The initial time point of chimerism analysis has differed among previous studies.[10-14] Conventionally, the first analysis of chimerism status has been performed approximately 1 month after allo-HSCT because the remaining recipient cells are frequently detected in the samples obtained too early after transplantation.[11,12,14-16] However, to the best of our knowledge, studies comparing transplant outcomes according to day 14 chimerism status after allo-HSCT in patients with non-malignant disease have not yet been reported. Therefore, the main aim of this study was to determine the impact of day 14 peripheral blood chimerism status on the transplant outcomes in patients who underwent allo-HSCT for non-malignant diseases.

METHODS

Patients

We retrospectively reviewed the medical records of 56 patients with non-malignant disease who received allo-HSCT at the Samsung Medical Center between April 2007 and March 2016. Non-malignant diseases included severe aplastic anemia, pure red cell aplasia, congenital dyserythropoietic anemia, severe congenital neutropenia, hemophagocytic lymphohistiocytosis, and primary immunodeficiency disease. The patients with X-linked severe combined immunodeficiency were excluded because they received allo-HSCT without conditioning.

Chimerism analyses

Chimerism analyses were performed using short-tandem repeat (STR) polymerase chain reaction (PCR). STR markers were evaluated in a given patient-donor pair using the fluorescence PCR technique. PCR was performed on the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA), and amplicon sizes were resolved by electrophoresis. One or two informative STR markers were selected for follow-up in a given patient-donor pair. Quantification of recipient DNA was performed by calculating areas under the peaks using GeneMapper software (Applied Biosystems) as previously described.[17] We regarded it as complete donor chimerism (CC) if ≥ 99% of the cells were of donor origin. MC was defined if > 1% of the cells were recipient-derived in the presence of donor-origin hematopoiesis which was further categorized into low-level MC (> 1% and < 15% of recipient-

derived cells) and high-level MC (≥ 15% of the recipient-derived cells). Chimerism status was evaluated at day 14 after allo-HSCT in peripheral blood and at 1 month in BM aspirates. Thereafter, chimerism status was evaluated at 3 months, 6 months, and 1 year post-transplant in BM aspirates.

Conditioning regimens and graft-versus-host disease (GVHD) prophylaxis

Patients received allo-HSCT with various myeloablative conditioning (MAC) or reduced-intensity conditioning (RIC) regimens depending on their disease or pre-transplant performance status. Prophylaxis against GVHD was based on cyclosporine (3 mg/kg per day) or tacrolimus (0.03 mg/kg per day) with or without short-term methotrexate, and mycophenolate mofetil replaced methotrexate for cord blood (CB) transplants. Initial drug levels were adjusted to maintain cyclosporine concentration within 200–300 ng/mL, and tacrolimus within 15–20 ng/mL.

Statistics

The probabilities of overall survival (OS) and event-free survival (EFS) rates were estimated by the Kaplan-Meier method, and comparisons between survival curves were performed using the log-rank test. OS was defined as the time from transplantation to death from any cause. Event was defined as death, primary engraftment failure, late rejection, or progression of primary disease, whichever occurred first. Primary engraftment failure was defined as failure to achieve a neutrophil count greater than 0.5×10^9/L after transplantation. Patient characteristics and clinical outcomes were compared using linear by linear association or Fisher's exact test for binary variables and Kruskal-Wallis test or Mann-Whitney U test for continuous variables. Pearson correlation analysis was used to observe the relationships between two continuous variables. Statistical differences were considered significant if $P < 0.05$.

Ethics statement

This study was approved by the Institutional Review Board (IRB) of the Samsung Medical Center (IRB No. SMC 2017-09-085). The need for informed consent was waived by the board.

RESULTS

Patients

Fifty-six patients with non-malignant disease underwent allo-HSCT with a median follow-up of 49 (range, 12–107) months, and the 5-year OS and EFS rates were 79.4% ± 5.6% and 75.8% ± 5.9%, respectively. Severe aplastic anemia (n = 25) was the most common disease followed by chronic granulomatous disease (n = 10), hemophagocytic lymphohistiocytosis (n = 6), Wiskott-Aldrich syndrome (n = 4), Fanconi anemia (n = 3), other BM failures (n = 5), and other primary immunodeficiency diseases (n = 3).

Chimerism

Because all 56 patients showed > 1% donor-derived hematopoiesis according to their day 14 peripheral blood chimerism analyses, they were able to be allocated into one of the 3 groups at day 14 post-transplant: CC (n = 36; 64.3%), low-level MC (n = 14; 25.0%), or high-level MC (n = 6; 10.7%). Of 36 patients with day 14 CC, 6 patients had MC at 1 month (donor range, 84.5%–98.8%), among whom 5 patients achieved CC again at 3 months and beyond until 1 year, while the remaining 1 patient who showed low-level MC (95.5% donor cells) at 1 month died due to septicemia at 3 months. Of 30 patients who showed CC at both day 14 and 1 month, 25 maintained
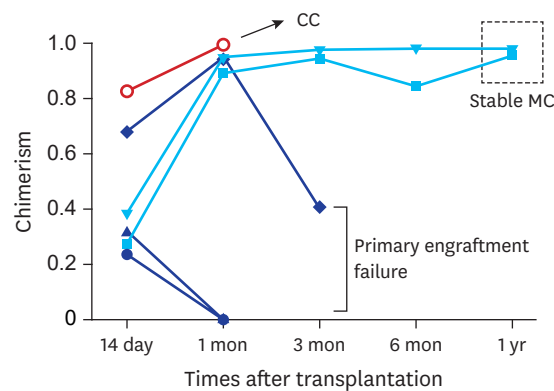
Fig. 1. Kinetics of chimerism in recipients with high-level MC.
CC = complete donor chimerism, MC = mixed chimerism.

CC until 1 year, 1 maintained CC until 6 months but then showed low-level MC (donor 98.8%) at 1 year. The remaining 4 patients died before the next evaluation of chimeric status.

Of the 14 patients with day 14 low-level MC (donor range, 85.6%–98.9%), 1 maintained stable MC (donor 97.9%) at 1 year, and 12 patients showed CC by 1 year; 5 achieved CC at 1 month, 4 at 4 months, 1 at 6 months, and 2 at 1 year. One patient with day 14 low-level MC (92.6% donor cells) who achieved CC at 3 months died due to systemic fungal infection at 4 months.

Of the 6 patients with day 14 high-level MC (donor range, 23.7%–82.7%), 3 patients (donor 23.7%, 31.6%, and 68.3%, respectively) failed primary engraftment, 1 patient achieved CC at 1 month but died due to cytomegalovirus pneumonia at 2 months, and 2 patients remained stable MC (donor 96.3% and 97.7%, respectively) at 1 year post-transplant. The chimeric status of patients with day 14 high-level MC is shown in **Fig. 1**.

Transplant characteristics and outcomes
Table 1 shows the differences in the transplant characteristics among the day 14 CC, low-level MC, and high-level MC groups. There were no statistical differences among the 3 groups in diseases, donors, and stem cell sources, but RIC regimens were more frequent in the day 14 CC or low-level MC group than in the day 14 high-level MC group (CC vs. high-level MC, 61.1% vs. 0%, $P = 0.007$; low-level MC vs. high-level MC, 50.0% vs. 0%, $P = 0.049$). There was a positive correlation between the degree of day 14 donor chimerism and the number of infused CD34$^+$ cells ($r = 0.302$, $P = 0.027$) (**Fig. 2**). **Table 2** shows the post-transplant outcomes according to the day 14 peripheral blood chimerism status. Neutrophil engraftment was faster in the day 14 CC group compared with that of the day 14 high-level MC group (12 vs. 16 days, $P = 0.006$). Notably, 3 patients in the day 14 high-level MC group but none in the day 14 CC or low-level MC group developed primary engraftment failure ($P < 0.001$). There was no difference in the incidence of grade II–IV acute GVHD ($P = 0.486$) or chronic GVHD ($P = 0.683$) among the 3 groups.

In the day 14 CC group, five deaths occurred caused by septic shock (n = 2), fungal pneumonia (n = 1), cytomegalovirus pneumonia (n = 1), and veno-occlusive disease (n = 1). In the day 14 low-level MC group, four deaths occurred caused by septic shock (n = 2) and chronic GVHD (n = 2). In the day 14 high-level MC group, 3 patients failed primary engraftment and one died of septic shock. Of the 3 patients with primary engraftment failure in the high-level MC group, 2 patients received second allo-HSCT and the remaining one patient died of septic
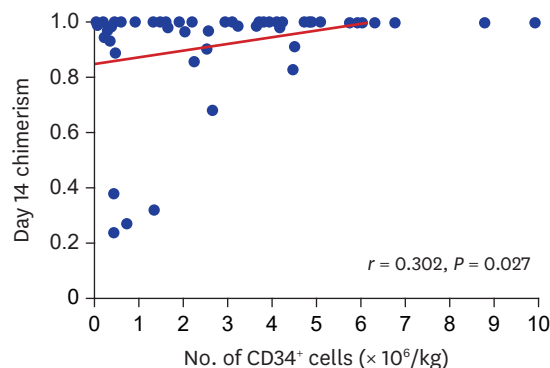
Table 1. Demographic and transplant characteristics according to day 14 chimerism status

Variables	CC (n = 36)	Low-level MC (n = 14)	High-level MC (n = 6)
Median age at transplantation, yr	10.3 (0.6–26.3)	5.3 (0.8–17.5)	4.5 (1.1–13.6)
Gender			
Male/female	25/11	10/4	5/1
Disease			
BM failures			
Severe aplastic anemia	18 (50.0)	7 (50.0)	0 (0)
Fanconi anemia	3 (8.3)	0 (0)	0 (0)
Other BM failures	4 (11.1)	0 (0)	1 (16.7)
Non-BM failures			
Chronic granulomatous disease	4 (11.1)	3 (21.4)	3 (50.0)
Hemophagocytic lymphohistiocytosis	4 (11.1)	1 (7.1)	1 (16.7)
Wiskott-Aldrich syndrome	1 (2.8)	3 (21.4)	0 (0)
Other primary immune deficiency diseases	2 (5.6)	0 (0)	1 (16.7)
Donor			
Matched related donor	5 (13.9)	2 (14.3)	0 (0)
Matched unrelated donor			
6/6 matched	5 (13.9)	3 (21.4)	0 (0)
8/8 matched	13 (36.1)	5 (35.7)	2 (33.3)
Mismatched unrelated donor	7 (19.4)	4 (28.6)	4 (66.7)
Haploidentical familial donor	6 (16.7)	0 (0)	0 (0)
Conditioning regimens			
Myeloablative	14 (38.9)	7 (50.0)	6 (100.0)
Reduced-intensity[a]	22 (61.1)	7 (50.0)	0 (0)
Stem cell source			
BM	4 (11.1)	1 (7.1)	0 (0)
PBSC	25 (69.4)	8 (57.1)	2 (33.3)
CB	7 (19.4)	5 (35.7)	4 (66.7)
GVHD prophylaxis			
CsA-based	21 (58.3)	7 (50.0)	4 (66.7)
Tacrolimus-based	15 (41.7)	7 (50.0)	2 (33.3)
Infused cells			
TNC (× 10^8/kg)	7.16 (0.34–18.26)	4.36 (0.73–16.25)	1.12 (0.11–8.31)
CD34$^+$ cells (× 10^6/kg)	4.00 (0.07–9.93)	2.15 (0.21–4.49)	1.04 (0.42–4.46)

Data are presented as median (range) or number (%).
CC = complete donor chimerism, MC = mixed chimerism, BM = bone marrow, PBSC = peripheral blood stem cell, CB = cord blood, GVHD = graft-versus-host disease, CsA = cyclosporine, TNC = total neutrophil count.
[a]The conventional conditioning regimens for severe aplastic anemia were regarded as reduced-intensity conditioning.

Fig. 2. Pearson correlation analysis between day 14 chimerism level and the number of infused CD34$^+$ cells.

shock. Although the 5-year OS was comparable among the day 14 CC, and low-level MC, and high-level MC groups (86.1% vs. 71.4% vs. 66.7%, $P = 0.371$) (**Fig. 3A**), the 5-year EFS was significantly higher in the day 14 CC or low-level MC group than in the day 14 high-level MC group (86.1% vs. 71.4% vs. 33.3%, $P = 0.001$) (**Fig. 3B**).

**Table 2.** Post-transplant outcomes according to day 14 chimerism status

Variables	CC (n = 36)	Low-level MC (n = 14)	High-level MC (n = 6)
Infectious complications			
Bacteremia	7 (19.4)	3 (21.4)	1 (16.7)
Invasive fungal infection	7 (19.4)	3 (21.4)	1 (16.7)
CMV disease	4 (11.1)	2 (14.3)	2 (33.3)
PTLD	1 (2.8)	2 (14.3)	0 (0)
Acute GVHD			
0–I	13 (36.1)	3 (21.4)	4 (66.7)
II–IV	23 (63.9)	11 (78.6)	2 (33.3)
III–IV	7 (19.4)	5 (35.7)	1 (16.7)
Chronic GVHD			
Limited	12 (33.3)	3 (21.4)	0 (0)
Extended	7 (19.4)	5 (35.7)	1 (16.7)
Engraftment			
Days to ANC 0.5×10^9/L	12 (9–25)	13 (10–19)	16 (13–22)
Days to PLT 20×10^9/L	18 (0–141)	18 (9–53)	38 (27–43)
Graft failure	0 (0)	0 (0)	3 (50.0)
Death	5 (13.9)	4 (28.6)	2 (33.3)

Data are presented as number (%).
CC = complete donor chimerism, MC = mixed chimerism, CMV = cytomegalovirus, PTLD = post-transplant lymphoproliferative disease, GVHD = graft-versus-host disease, ANC = absolute neutrophil count, PLT = platelet.

Outcomes according to graft source

The primary engraftment failure rates according to the graft source showed 0% (0/5) with BM, 2.9% (1/35) with peripheral blood stem cells (PBSC), and 12.5% (2/16) with CB. The median times to neutrophil and platelet engraftment were faster in BM/PBSC transplants than in CB transplants (neutrophils: 12 vs. 17 days, $P < 0.001$; platelets: 17 vs. 42 days, $P = 0.001$, respectively). The 5-year OS and EFS rates were not statistically different between donor graft sources (OS: BM/PBSC 83.1% vs. CB 68.8%, $P = 0.160$; EFS: BM/PBSC 80.6% vs. CB 68.8%, $P = 0.082$, respectively). In transplants with a BM/PBSC graft, although the 5-year OS was comparable among the day 14 CC, low-level MC, and high-level MC groups (93.1% vs. 66.7% vs. 50.0%, $P = 0.060$) (**Fig. 4A**), the 5-year EFS was significantly higher in the day 14 CC or low-level MC group than in the day 14 high-level MC group (93.1% vs. 66.7% vs. 0%, P
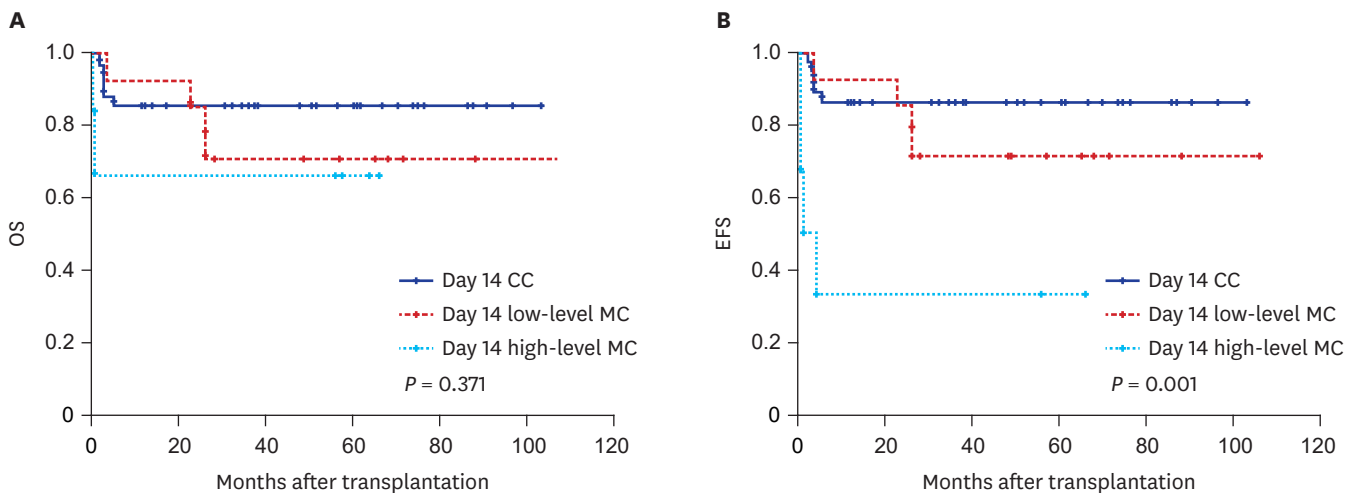
Fig. 3. OS and EFS rates according to day 14 chimerism status. All P values were determined by the log-rank test. (**A**) The 5-year OS were similar among the day 14 CC and low- and high-level MC groups (86.1% vs. 71.4% vs. 66.7%, $P = 0.371$). (**B**) The 5-year EFS was higher in patients with CC or low-level MC than those with high-level MC (86.1% vs. 71.4% vs. 33.3%, $P = 0.001$).
OS = overall survival, EFS = event-free survival, CC= complete donor chimerism, MC = mixed chimerism.
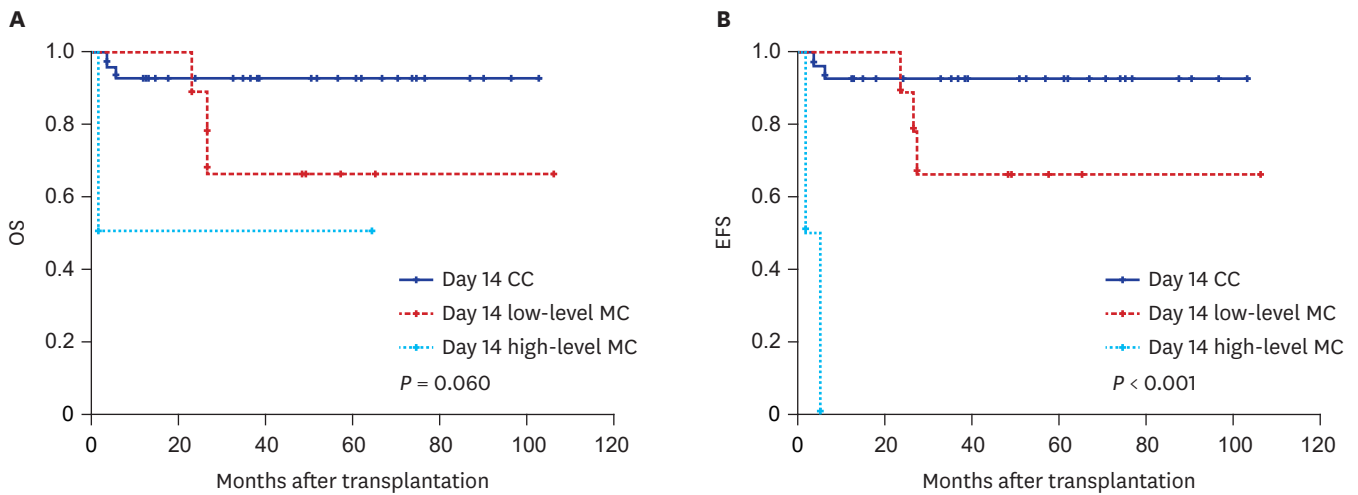
Fig. 4. OS and EFS rates according to day 14 chimerism status in transplants with BM/PBSC. (**A**) The 5-year OS were comparable among the day 14 CC and low- and high-level MC groups (93.1% vs. 66.7% vs. 50.0%, P = 0.060). (**B**) The 5-year EFS was higher in patients with CC or low-level MC groups than those with high-level MC (93.1% vs. 66.7% vs. 0%, P < 0.001).
OS = overall survival, EFS = event-free survival, BM/PBSC = bone marrow or peripheral blood stem cell, CC = complete donor chimerism, MC = mixed chimerism.

< 0.001) (**Fig. 4B**). However, in CB transplants, the 5-year OS and EFS rates according to the day 14 peripheral blood chimerism did not reach statistical significance (OS: 57.1% vs. 80.0% vs. 75.0%, P = 0.662; EFS: 57.1% vs. 80.0% vs. 50.0%, P = 0.489).

DISCUSSION

Allo-HSCT has been a curative treatment modality for various non-malignant diseases. However, graft rejection has been an important problem in allo-HSCT for non-malignant diseases in which immune function is active and there was no previous chemotherapy, for which even MAC regimens may be insufficient to achieve CC.[18] Therefore, monitoring hematopoietic chimerism after allo-HSCT is important for assessing engraftment and graft rejection in non-malignant diseases.[19,20] Previous studies revealed that the early analysis of chimerism after allo-HSCT could be used to predict graft rejection, but the earliest time point of chimerism analysis varied among these studies.[10,20-22] Therefore, in this study, we aimed to determine whether the analysis of peripheral blood chimerism status at day 14 could help predict transplant outcomes including graft rejection in patients who underwent allo-HSCT for non-malignant diseases.

In this study, we found that the 5-year EFS was higher in the day 14 CC or low-level MC group than in the day 14 high-level MC group among BM/PBSC transplants, whereas there were no statistical differences in survival rates according to day 14 chimerism status in CB transplants. This might be explained by the difference in engraftment kinetics between BM/PBSC and CB transplants. In this study, hematologic recovery was delayed in CB transplants compared with BM/PBSC transplants, which might be associated with a lower infused number of CD34+ cells in the CB transplants than in the BM/PBSC transplants. Given that the median time to neutrophil recovery in CB transplants was 17 days in our cohort, day 14 may be too early for the chimerism status to have an impact on the transplant outcomes. On the contrary, hematologic recovery after BM/PBSC transplants was relatively faster enough for the early chimerism to be served as a predictor of transplant outcomes.

Full donor chimerism, in general, is not essential for curing non-malignant diseases because stable MC is frequently adequate for functional correction of the underlying disease.[18] However, as MC may progress to graft rejection, determining post-transplant chimerism could help identify patients at an increased risk of graft rejection and could be used as a guide for initiating active interventions.[1,19] In the current study, all of the primary engraftment failures occurred in patients with day 14 high-level MC, which suggests that those patients are at greater risk of graft loss and require a prompt intervention such as rapid reduction of immunosuppression. Previous studies also showed that high-level MC correlated with an increased risk of rejection.[10,13,16] Breuer et al.[10] reported that a high level of recipient-derived T and NK cells heralded graft loss in pediatric patients undergoing allo-HSCT. In a study by Ozyurek et al.,[13] 33% of children with non-malignant diseases who underwent allo-HSCT showed increasing MC up to 30% of recipient-derived cells and they were at a greater risk of graft loss. Similar findings were noted in a study by Svenberg et al.[16] in which graft rejection was found exclusively among patients with high-level MC. On the other hand, none of the patients with day 14 CC/low-level MC experienced primary engraftment failure in the present study. This result is similar to that of a previous study in which patients who showed CC or low-level MC at 1 month after allo-HSCT indicated low risk for graft rejection.[22] Notwithstanding the previous studies, we proved that earlier assessment of chimerism status using recipients' peripheral blood is also useful, which enables us to intervene more promptly following BM/PBSC transplants.

In the current study, we observed that the number of infused $CD34^+$ cells was positively correlated with the degree of day 14 donor chimerism. Although a study by Lawler et al.[11] showed that there was no correlation between the number of cells infused and chimerism status in allo-HSCT for severe aplastic anemia, other studies revealed that a higher number of infused $CD34^+$ cells was associated with CC.[20,23-26] Perez-Simon et al.[26] reported that 100% of patients receiving a higher dose of $CD34^+$ cells reached CC by days 21–28, but only 44% of patients who received a lower dose of $CD34^+$ cells achieved CC. Another study showed that a greater number of $CD34^+$ cells was associated with higher levels of donor chimerism and rapid achievement of CC on day 28.[23] Our results are in line with those reports showing that a higher $CD34^+$ cell dose is associated with a higher probability of achieving better chimerism. Further studies are required to determine the optimal number of $CD34^+$ cells needed to improve the outcomes.

Several studies have used RIC regimens in allo-HSCT for non-malignant diseases.[4,7,27-29] In one of these studies, Marsh et al.[7] reported that MC developed more frequently in the RIC group than in the MAC group (65% vs. 18%, $P = 0.011$) in patients with hemophagocytic lymphohistiocytosis. In another study, 55 patients with non-malignant disease underwent allo-HSCT with RIC or MAC regimens, and 41% of patients in the RIC group, but 66% of those in the MAC group, achieved CC ($P = 0.01$).[29] However, RIC regimens were more frequently used in the day 14 CC and low-level MC groups than in the high-level MC group in the current study. This may partly be explained by the fact that we regarded conventional conditioning regimens used for severe aplastic anemia as RIC which practically had myeloablative effects for those patients. In addition, because we used various conditioning regimens depending on patients' underlying diseases or pre-transplant performance status, we were not able to conclude the association between day 14 chimerism status and conditioning intensity in this study.

Our study has several limitations such as its retrospective nature, a relatively small population, and heterogeneity in the underlying diseases and transplant types. Nevertheless,

given the rarity of transplant candidates with non-malignant disease in the pediatric population, our results may provide useful insight about the impact of early chimerism on the transplant outcomes in pediatric non-malignant diseases.

In conclusion, our data suggest that analysis of post-transplant peripheral blood chimerism on day 14 may predict transplant outcomes in patients with non-malignant diseases after BM/PBSC transplantation. In CB transplants, however, the earliest clinically relevant time point should be defined in further studies. Larger multicenter prospective studies are required to verify the relationship between early chimerism and transplant outcomes.

REFERENCES

1. Willasch A, Hoelle W, Kreyenberg H, Niethammer D, Handgretinger R, Lang P, et al. Outcome of allogeneic stem cell transplantation in children with non-malignant diseases. *Haematologica* 2006;91(6):788-94.
   PUBMED

2. Ringdén O, Remberger M, Svahn BM, Barkholt L, Mattsson J, Aschan J, et al. Allogeneic hematopoietic stem cell transplantation for inherited disorders: experience in a single center. *Transplantation* 2006;81(5):718-25.
   PUBMED | CROSSREF

3. Mussetti A, Kernan NA, Prockop SE, Scaradavou A, Lehrman R, Ruggiero JM, et al. Allogeneic hematopoietic stem cell transplantation for nonmalignant hematologic disorders using chemotherapy-only cytoreductive regimens and T-cell-depleted grafts from human leukocyte antigen-matched or -mismatched donors. *Pediatr Hematol Oncol* 2016;33(6):347-58.
   PUBMED | CROSSREF

4. Klein OR, Chen AR, Gamper C, Loeb D, Zambidis E, Llosa N, et al. Alternative-donor hematopoietic stem cell transplantation with post-transplantation cyclophosphamide for nonmalignant disorders. *Biol Blood Marrow Transplant* 2016;22(5):895-901.
   PUBMED | CROSSREF

5. Bader P, Beck J, Frey A, Schlegel PG, Hebarth H, Handgretinger R, et al. Serial and quantitative analysis of mixed hematopoietic chimerism by PCR in patients with acute leukemias allows the prediction of relapse after allogeneic BMT. *Bone Marrow Transplant* 1998;21(5):487-95.
   PUBMED | CROSSREF

6. Bader P, Dückers G, Kreyenberg H, Hoelle W, Kerst G, Lang P, et al. Monitoring of donor cell chimerism for the detection of relapse and early immunotherapeutic intervention in acute lymphoblastic leukemias. *Ann Hematol* 2002;81 Suppl 2:S25-7.
   PUBMED

7. Marsh RA, Vaughn G, Kim MO, Li D, Jodele S, Joshi S, et al. Reduced-intensity conditioning significantly improves survival of patients with hemophagocytic lymphohistiocytosis undergoing allogeneic hematopoietic cell transplantation. *Blood* 2010;116(26):5824-31.
   PUBMED | CROSSREF

8. Andreani M, Manna M, Lucarelli G, Tonucci P, Agostinelli F, Ripalti M, et al. Persistence of mixed chimerism in patients transplanted for the treatment of thalassemia. *Blood* 1996;87(8):3494-9.
   PUBMED

9. Thiede C. Diagnostic chimerism analysis after allogeneic stem cell transplantation: new methods and markers. *Am J Pharmacogenomics* 2004;4(3):177-87.
   PUBMED | CROSSREF

10. Breuer S, Preuner S, Fritsch G, Daxberger H, Koenig M, Poetschger U, et al. Early recipient chimerism testing in the T- and NK-cell lineages for risk assessment of graft rejection in pediatric patients undergoing allogeneic stem cell transplantation. *Leukemia* 2012;26(3):509-19.
    PUBMED | CROSSREF

11. Lawler M, McCann SR, Marsh JC, Ljungman P, Hows J, Vandenberghe E, et al. Serial chimerism analyses indicate that mixed haemopoietic chimerism influences the probability of graft rejection and disease recurrence following allogeneic stem cell transplantation (SCT) for severe aplastic anaemia (SAA): indication for routine assessment of chimerism post SCT for SAA. *Br J Haematol* 2009;144(6):933-45.
    PUBMED | CROSSREF

12. Oshrine BR, Olson TS, Bunin N. Mixed chimerism and graft loss in pediatric recipients of an alemtuzumab-based reduced-intensity conditioning regimen for non-malignant disease. *Pediatr Blood Cancer* 2014;61(10):1852-9.
PUBMED | **CROSSREF**

13. Ozyurek E, Cowan MJ, Koerper MA, Baxter-Lowe LA, Dvorak CC, Horn BN. Increasing mixed chimerism and the risk of graft loss in children undergoing allogeneic hematopoietic stem cell transplantation for non-malignant disorders. *Bone Marrow Transplant* 2008;42(2):83-91.
PUBMED | **CROSSREF**

14. Rao K, Adams S, Qasim W, Allwood Z, Worth A, Silva J, et al. Effect of stem cell source on long-term chimerism and event-free survival in children with primary immunodeficiency disorders after fludarabine and melphalan conditioning regimen. *J Allergy Clin Immunol* 2016;138(4):1152-60.
PUBMED | **CROSSREF**

15. Dubovsky J, Daxberger H, Fritsch G, Printz D, Peters C, Matthes S, et al. Kinetics of chimerism during the early post-transplant period in pediatric patients with malignant and non-malignant hematologic disorders: implications for timely detection of engraftment, graft failure and rejection. *Leukemia* 1999;13(12):2059.
PUBMED | **CROSSREF**

16. Svenberg P, Mattsson J, Ringdén O, Uzunel M. Allogeneic hematopoietic SCT in patients with non-malignant diseases, and importance of chimerism. *Bone Marrow Transplant* 2009;44(11):757-63.
PUBMED | **CROSSREF**

17. Park CH, Song MJ, Kim HJ, Ki CS, Lee SH, Yoo KH, et al. Underestimation of recipient DNA in bone marrow by post-transplant chimerism analyses using DNA extracted from EDTA-collected aspirate samples in pediatric acute myeloid leukemia. *Ann Clin Lab Sci* 2011;41(2):138-43.
PUBMED

18. Antin JH, Childs R, Filipovich AH, Giralt S, Mackinnon S, Spitzer T, et al. Establishment of complete and mixed donor chimerism after allogeneic lymphohematopoietic transplantation: recommendations from a workshop at the 2001 Tandem Meetings of the International Bone Marrow Transplant Registry and the American Society of Blood and Marrow Transplantation. *Biol Blood Marrow Transplant* 2001;7(9):473-85.
PUBMED | **CROSSREF**

19. Hoelle W, Beck JF, Dueckers G, Kreyenberg H, Lang P, Gruhn B, et al. Clinical relevance of serial quantitative analysis of hematopoietic chimerism after allogeneic stem cell transplantation in children for severe aplastic anemia. *Bone Marrow Transplant* 2004;33(2):219-23.
PUBMED | **CROSSREF**

20. Park M, Koh KN, Seo JJ, Im HJ. Clinical implications of chimerism after allogeneic hematopoietic stem cell transplantation in children with non-malignant diseases. *Korean J Hematol* 2011;46(4):258-64.
PUBMED | **CROSSREF**

21. Ketterl TG, Flesher M, Shanley R, Miller W. Early CD3+/CD15+ peripheral blood leukocyte chimerism patterns correlate with long-term engraftment in non-malignant hematopoietic SCT. *Bone Marrow Transplant* 2014;49(4):572-5.
PUBMED | **CROSSREF**

22. Mellgren K, Arvidson J, Toporski J, Winiarski J. Chimerism analysis in clinical practice and its relevance for the detection of graft rejection and malignant relapse in pediatric hematopoietic stem cell transplant patients. *Pediatr Transplant* 2015;19(7):758-66.
PUBMED | **CROSSREF**

23. Baron F, Maris MB, Storer BE, Sandmaier BM, Panse JP, Chauncey TR, et al. High doses of transplanted CD34+ cells are associated with rapid T-cell engraftment and lessened risk of graft rejection, but not more graft-versus-host disease after nonmyeloablative conditioning and unrelated hematopoietic cell transplantation. *Leukemia* 2005;19(5):822-8.
PUBMED | **CROSSREF**

24. Gonzalez-Vicent M, Diaz MA. Higher doses of CD34+ PBPC are associated with a rapid acquisition of full donor chimerism and lower risk of relapse after allogeneic transplantation in pediatric patients with hematological malignancies. *J Pediatr Hematol Oncol* 2011;33(3):185-9.
PUBMED | **CROSSREF**

25. Mickelson DM, Sproat L, Dean R, Sobecks R, Rybicki L, Kalaycio M, et al. Comparison of donor chimerism following myeloablative and nonmyeloablative allogeneic hematopoietic SCT. *Bone Marrow Transplant* 2011;46(1):84-9.
PUBMED | **CROSSREF**

26. Perez-Simon JA, Diez-Campelo M, Martino R, Sureda A, Caballero D, Canizo C, et al. Impact of CD34+ cell dose on the outcome of patients undergoing reduced-intensity-conditioning allogeneic peripheral blood stem cell transplantation. *Blood* 2003;102(3):1108-13.
PUBMED | **CROSSREF**

27. Parikh SH, Mendizabal A, Benjamin CL, Komanduri KV, Antony J, Petrovic A, et al. A novel reduced-intensity conditioning regimen for unrelated umbilical cord blood transplantation in children with nonmalignant diseases. *Biol Blood Marrow Transplant* 2014;20(3):326-36.
PUBMED | **CROSSREF**

28. Marsh RA, Rao MB, Gefen A, Bellman D, Mehta PA, Khandelwal P, et al. Experience with alemtuzumab, fludarabine, and melphalan reduced-intensity conditioning hematopoietic cell transplantation in patients with nonmalignant diseases reveals good outcomes and that the risk of mixed chimerism depends on underlying disease, stem cell source, and alemtuzumab regimen. *Biol Blood Marrow Transplant* 2015;21(8):1460-70.
PUBMED | **CROSSREF**

29. Ringdén O, Remberger M, Svenberg P, Svahn BM, Dahllöf G, Gustafsson B, et al. Fludarabine-based disease-specific conditioning or conventional myeloablative conditioning in hematopoietic stem cell transplantation for treatment of non-malignant diseases. *Bone Marrow Transplant* 2007;39(7):383-8.
PUBMED | **CROSSREF**