RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2021-215
Cyprus J Med Sci 2022;7(5):664-668

Chimerism Analysis of Children with Allogeneic Stem-Cell transplantation and Its Effect on Survival

Hakan Tekgüç[1], Serap Aksoylar[2], Doğa Ceren Tekgüç[3], Savaş Kansoy[2]

[1]Department of Pediatric Intensive Care, Dr. Burhan Nalbantoglu State Hospital, Nicosia, North Cyprus
[2]Department of Child Health and Diseases, Ege University Faculty of Medicine, İzmir, Turkey
[3]Department of Developmental and Behavioral Pediatrics, Dr. Burhan Nalbantoglu State Hospital, Nicosia, Cyprus

Abstract

BACKGROUND/AIMS: Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is an important and usually the only curative clinical tool for treating pediatric patients with many hereditary and acquired diseases. Although complete donor stem cell engraftment is the desired result of Allo-HSCT, patients do not always have a definite engraftment and end up with mixed chimerism. Many factors both related to patient and transplantation can affect chimerism levels. Additionally, mixed chimerism levels may affect the event free survival (EFS) differently in distinct diseases. The major goals of this study were to determine the first 100-day donor chimerism ratios and to search for a relationship between donor chimerism success (CS) (for malignant diseases, hematopoietic donor chimerism >95%; for non-malignant diseases, >70%) and EFS for pediatric patients.

MATERIALS AND METHODS: We collected data from 95 pediatric patients who underwent Allo-HSCT between March, 2005 and April, 2010 at Ege University Hospital with at least one chimerism result obtained within the first 100 days.

RESULTS: After checking for all other factors, CS in the first 100 days increases the chance of post-transplant EFS by -3.04 (-4.00 to -2.08) [hazard ratio (HR): 0.05 ($p<0.001$)]. Neutrophil engraftment was the other factor which was positively correlated with EFS (HR p-value: 0.05)

CONCLUSION: There is a positive correlation between CS in the first 100 days and EFS for both malignant and non-malignant diseases.

Keywords: Children, chimerism, stem-cell-transplantation

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the only curative treatment for many congenital and acquired, malignant and non-malignant diseases of the childhood period.[1,2] Donor stem cell engraftment is a critical step in the success of Allo-HSCT.[3] However, engraftment does not always result in a definite status and we may end up with mixed chimerism. Mixed chimerism is a state where both the recipient and donor hematopoietic stem cells coexist in the host's bone marrow.[4] Although malignancies require a full donor match (>95%), mixed chimerism may cure primary immunodeficiency and hemoglobinopathy.[5,6] Therefore, chimerism success (CS) for malignant diseases requires >95% donor cells, while for non-malignant diseases, a donor cell ratio of over 70% can be accepted as success. Many factors affect survival but the chimerism status of the first 100 days is a useful parameter to predict survival.[7] Understanding the factors and elements associated with CS in the first 100 days will help to improve overall survival (OS) in HSCT.[8] Serial analysis of the patient's chimerism levels at fixed time points and on suspicion of relapse or graft failure may be used to track engraftment levels, disease control, and relapse risk.[9]

With this study, we aimed to understand the factors affecting CS and event free survival (EFS) after Allo-HSCT in a mixed group of pediatric patients.

To cite this article: Tekgüç H, Aksoylar S, Tekgüç DC, Kansoy S. Chimerism Analysis of Children with Allogeneic Stem-Cell transplantation and Its Effect on Survival. Cyprus J Med Sci 2022;7(5):664-668

ORCID IDs of the authors: H.T. 0000-0001-6424-6761; S.A. 0000-0002-8446-0834; D.C.T. 0000-0003-4536-9365; S.K. 0000-0003-0799-9518.

Address for Correspondence: Hakan Tekgüç
E-mail: tekguchakan@gmail.com
ORCID ID: orcid.org/0000-0001-6424-6761

Received: 11.08.2021
Accepted: 15.04.2022

MATERIALS AND METHODS

We retrospectively reviewed pediatric patients who had undergone allogeneic HSCT transplantation at Ege University and had at least one chimerism result obtained within the first 100 days. The Ege University Ethics Committee approved this study which was executed according to the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards (approval number: 07-5.1-14, date: 08.08.2007). Chimerism statuses were assayed from bone marrow for the first month and peripheral blood for the second and third months. We defined CS as >95% and >70% for malignant diseases and non-malignant diseases, respectively. We investigated associations between CS with patient demographics, indications for HSCT, donor/graft, and post-transplantation factors. After DNA extraction was performed from samples, data about chimerism were collected using polymerase chain reaction (PCR) amplification of short tandem repeats of 15 polymorphic loci and one amelogenin with the AmpFlSTR® Identifiler® PCR Amplification Kit.

Statistical Analysis

For these collected nominal variables, univariate survival analysis was performed according to the Kaplan-Meier method and two-tailed log-rank analysis was used to detect statistically significant differences.[10] Parameters with p-values of <0.1 were included in the multivariate survival analysis. The Cox proportional hazards model was used for both univariate and multivariate analysis for continuous parameters. Probit regression analysis was used to investigate the effective causes of the success of chimerism. As a result of the evaluation of all independent parameters in this study, statistically insignificant parameters were removed and the Probit model was repeated.[11] Analyses were performed using Stata/SE 14.0.

RESULTS

In this retrospective study, we analyzed 95 pediatric patients who had undergone Allo-HSCT between March, 2005 and April, 2010 and had at least one chimerism result obtained within the first 100 days. The median age was 102 months and 58 (61.0%) were male. Leukemia (28%), thalassemia major (24%), and diseases associated with bone marrow failure (25%) were the most common reasons for undergoing HSCT. Most of the donors were matched sibling donors (Table 1). In 33 cases (35%), transplantation was from women to men, and the stem cell source was predominantly peripheral blood (58%). In three patients (3.2%) with severe combined immune deficiency, no conditioning regimen was used. A myeloablative conditioning regimen was used in 68.4% of patients, and non-myeloablative regimens were used in 28.4%.

Table 1. Transplantation characteristics

Char.	Groups		Patient number (n)	%	n	Range
Donor and HLA match (*)	MSD		75	79		
	MRD		9	10		
	MMRD		5	5		
	MUD		6	6		
Donor/recipient sex	M	M	25	26		
	F	F	19	20		
	M	F	17	18		
	F	M	33	35		
Stem cell source	Peripheral blood		55	58		
	Bone marrow		30	42		
	Cord blood		1	1		
	Bone marrow + cord blood		8	8		
Conditioning regimen	None		3	3		
	Myeloablative		65	68		
	Non-myeloablative		27	28		
CD34 (+) cell count (x10^6/kg)						
Median					5	0.2-44.4
GvHD prophylaxis	CSA		30	32		
	CSA + MTX		57	60		
	CSA + MMF		1	1		
	No prophylaxis		7	7		
WC engraftment						
(ANS ≥500/mm^3)			88	93		
(A)GvHD	Grade 0-1		72	76		
	Grade 2-4		23	24		
Disease	Relapsed		21	22		
	No relapsed		74	78		

MSD: matched sibling donor, MRD: matched related donor, MMRD: mismatched related donor, MUD: matched unrelated donor, M: male, F: female, GvHD: graft versus host disease, CSA: cyclosporine, MTX: methotrexate, MMF: mycophenolate mofetil, WC: white blood cell, (A)GvHD: (acute) graft versus host disease.

Table 2. Relation between patient and HSCT characteristics and event-free survival	
Patient or SCT characteristics	**p-value**
Sex	
Male/female	0.86
Age	0.16
Primary disease	
Malignant/non-malignant	0.24
Donor and HLA match	
MSD/MRD/MMRD/MUD	**0.01**
Host and donor sex differences	0.06
Stem cell source	
Peripheral blood, bone marrow, cord blood, bone marrow and cord blood	0.94
Conditioning regimen	
Myeloablative/non-myeloablative-no conditioning	0.32
CD34 (+) cell count (median, x10^6/kg)	
≥5/<5	0.05
Neutrophil engraftment	
Existing/absent	**<0.001**
(A)GvHD	
Existing/absent	0.27
VOD	
Existing/absent	0.32
First 100 days chimerism success	
Existing/absent	**<0.001**

HSCT: hematopoietic stem cell transplantation, SCT: stem cell transplantation, HLA: human leukocyte antigen, MSD: matched sibling donor, MRD: matched related donor, MMRD: mismatched related donor, MUD: matched unrelated donor, (A)GvHD: (acute) graft versus host disease, VOD: veno occlusive disease.

The median CD34+ cell count was 5x10^6 cells/kg (minimum: 0.27x10^6/kg, maximum: 44.4x10^6/kg). For graft versus host disease (GvHD) prophylaxis, 31.6% of patients received cyclosporine (CSA) only. In 56.8% of patients, CSA was combined with methotrexate, and in one patient, CSA was combined with mycophenolate mofetil.

In 92.6% of patients, neutrophil engraftment was observed between the 9th-36th (median 17th) days of HSCT. 24.2% of patients were diagnosed as having grade 2-4 acute GvHD. Veno-occlusive disease (VOD) was detected in eight patients (8.4%). 30.5% of patients either had a relapse of their primary disease or died of HSCT complications. 8.4% of patients died after SCT without relapse. In 22.1% of patients, primary disease relapse was detected, seven of whom died of disease relapse, the latest being at the 18th month of SCT. With a mean of 22 months of follow-up, the OS was 84.0% and even EFS was 69.5%.

We excluded four patients from the further analysis. Three of them were excluded from the analysis because they could not complete the first 100 days after SCT and one patient was re-transplanted within the first 100 days. Of these 91 patients, 77 (84.6%) had CS within the first 100 days. The CS was 84.4% and 84.7% in patients with malignant and non-malignant diseases, respectively.

We evaluated factors which affect EFS and our univariate analysis revealed that CS within the first 100 days, human leukocyte antigen (HLA) matched donors, the amount of CD34+ cells (over 5x10^6), and neutrophil engraftment were associated with EFS (Table 2). The patients' age, sex, primary disease type of the conditioning regimen, stem cell source, and the existence of GvHD or VOD were found to be unrelated to EFS. Parameters with p-values <0.1 were evaluated using multivariate analysis (Table 3). In multivariate survival analysis, we found that CS within the first 100 days, neutrophil engraftment, and female to female SCT were found to be related to EFS.

Discussion

Allo-HSCT is the only curative treatment method in many congenital or acquired childhood diseases.[12,13] Most of these diseases are acute leukemia and congenital diseases such as congenital bone marrow deficiencies (Fanconi anemia), hemoglobin synthesis defects (thalassemia major), congenital immunodeficiency, and some metabolic diseases (osteopetrosis), which are common in Turkey where consanguineous marriage is still widespread.[1] The fact that the primary diseases of the patients included in this study were extremely heterogeneous, the primary diseases were either malignant or non-malignant, and the patients who received myeloablative or non-myeloablative treatment were in the same pool made the evaluation difficult.

In this study, due to the shorter follow-up period compared with similar studies, instead of evaluating increasing, decreasing, and stable mixed chimerism,[13] CS within the first 100 days was evaluated as per Holtan et al.[14]. In the present study, 77 (84.6%) out of 91 patients achieved CS within the first 100 days, which is slightly higher than the findings of Ünal İnce et al.[15] regarding T. Major patients transplanted between 1999-2007 in Ankara, Turkey. Their rate was 84.4% in malignant diseases and 84.7% in non-malignant diseases. Although this rate varies according to the type of primary disease (malignant, non-malignant) and the type of conditioning regimen used (myeloablative, non-myeloablative), it was found to be 64% in the study of Holtan et al.[14].

Similar to the literature,[16,17] in univariate analysis, HLA-matched donor selection had a positive effect on EFS. However, multivariate analysis

Table 3. Multivariate analysis of parameters related with event free survival				
Independent variables	**RR**	**95% CI**	**HR**	**HR p-value**
First 100 days chimerism success	**-3.0432**	**(-4.0041 to -2.0824)**	**0.05**	**<0.001**
Existing neutrophil engraftment	**-0.8010**	**(-1.5876 to -0.0143)**	**0.45**	**0.05**
Female donor to female recipient	-2.1620	(-4.5000 to 0.1761)	0.12	0.07
HLA matched donor	-0.41	(-1.2684 to 0.4442)	0.66	0.28
CD34+ cell count over ≥5x10^6/kg	-0.12	(-1.1710 to 0.9216)	0.88	0.82

RR: risk ratio, CI: confidence interval, HR: hazard ratio.

showed no statistically meaningful effect. Again, in multivariate analysis, no difference was found when the donor's HLA-matched sibling was compared with other donor types. We think that no statistically significant results were obtained in the multivariate analysis due to the low number of transplants from unrelated donors in our patient group and the lack of long-term follow-up of these patients. It should be noted that in the study published by Holtan et al.[14] in 2010, donor type and HLA match were also found to have no positive effect on EFS. Contrary to the literature, in our study, CD34+ cell counts above the median value had no statistically significant effect on survival.[18]

Unlike the literature, in our study, the gender relationship between the donor and recipient was found to be associated with EFS.[19] Randolph et al.[20] showed that male recipients of female transplants had the lowest risk for relapse and the greatest odds for GVHD, however in our study, it was observed that female-to-female transplants had a positive effect on survival compared with the other groups. Regardless of all other parameters, the realization of neutrophil engraftment in the recipient positively affected EFS.

It was observed that the most important factor which affected EFS was CS within the first 100 days. The risk of recurrence and death decreased with CS within the first 100 days [hazard ratio: 0.05 ($p<0.001$)]. In previous studies on hematologic malignancies, 95% and above donor chimerism was shown to have a positive effect on EFS.[21] Our study suggests that donor chimerism of 70% or higher within the first 100 days may also have a positive effect on survival in non-malignant diseases.

Study Limitations

The low number of patients and the shortness of the follow-up period were the major limitations of this study. With more patients, longer follow-up and more chimerism results, we would be able to understand more about the relationship between chimerism and disease relapse in malignancy, and graft loss in non-malignant diseases. Although a significant amount of time has passed since the time of our study, the effects of chimerism on EFS have still not been clarified in the literature.[22]

CONCLUSION

The success of chimerism was found satisfactory in this heterogeneous patient group and CS within the first 100 days was the most important predictor of EFS.

MAIN POINTS

- In a heterogeneous group of pediatric Allo-HSCT patients, Chimerism Success within the first 100 days was the most important predictor of EFS.

ETHICS

Ethics Committee Approval: The Ege University Ethics Committee approved this study which was executed according to the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards (approval number: 07-5.1-14, date: 08.08.2007).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: H.T., Design: H.T., S.A., D.C.T., S.K., Supervision: H.T., S.A., S.K., Materials: H.T., Data Collection and/or Processing: H.T., S.A., D.C.T., Analysis and/or Interpretation: H.T., S.A., Literature Search: H.T., Writing: H.T., S.A., D.C.T., Critical Review: S.A., S.K.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The author declared that this study had received no financial support.

REFERENCES

1. Arat M, Arpaci F, Ertem M, Gürman G; Turkish Transplant Registry. Turkish Transplant Registry: a comparative analysis of national activity with the EBMT European Activity Survey. Bone Marrow Transplant. 2008; 42(1): S142-5.
2. Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Giardini C, et al. Bone marrow transplantation in patients with thalassemia. N Engl J Med. 1990; 322(7): 417-21.
3. Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med. 2006; 354(17): 1813-26.
4. Muller-Bérat N, Lion T. Chimerism and transplant-related diagnostics. Leukemia. 2006; 20(8): 1358-60.
5. Gardiner N, Lawler M, O'riordan J, De'arce M, Mccann SR. Donor chimaerism is a strong indicator of disease free survival following bone marrow transplantation for chronic myeloid leukaemia. Leukemia. 1997; 11(Suppl 3): 515-5.
6. Lucarelli G, Andreani M, Angelucci E. The cure of thalassemia by bone marrow transplantation. Blood Rev. 2002; 16(2): 81-5.
7. Wustrau K, Greil J, Sykora KW, Albert MH, Burkhardt B, Lang P, et al. Risk factors for mixed chimerism in children with hemophagocytic lymphohistiocytosis after reduced toxicity conditioning. Pediatric Blood Cancer. 2020; 67(9): e28523.
8. Peterlin P, Delaunay J, Guillaume T, Gastinne T, Mahé B, Dubruille V, et al. Complete donor T cell chimerism predicts lower relapse incidence after standard double umbilical cord blood reduced-intensity conditioning regimen allogeneic transplantation in adults. Biol Blood Marrow Transplant. 2015; 21(1): 180-4.
9. Ahci M, Stempelmann K, Buttkereit U, Crivello P, Trilling M, Heinold A, et al. Clinical utility of quantitative PCR for chimerism and engraftment monitoring after allogeneic stem cell transplantation for hematologic malignancies. Biol Blood Marrow Transplant. 2017; 23(10): 1658-68.
10. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958; 53(282): 457-81.
11. Finney DJ. Probit analysis, 3rd ed. Cambridge University Press, New York, London; 1971.
12. Möricke A, Zimmermann M, Reiter A, Henze G, Schrauder A, Gadner H, et al. Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia. 2010; 24(2): 265-84.
13. Svenberg P, Mattsson J, Ringden O, Uzunel M. Allogeneic hematopoietic SCT in patients with non-malignant diseases, and importance of chimerism. Bone Marrow Transplant. 2009; 44(11): 757-63.
14. Holtan SG, Hogan WJ, Elliott MA, Ansell SM, Inwards DJ, Porrata LF, et al. CD34+ cell dose and establishment of full donor chimerism at day +100 are important factors for survival with reduced-intensity conditioning

with fludarabine and melphalan before allogeneic hematopoietic SCT for hematologic malignancies. Bone Marrow Transplant. 2010; 45(12): 1699-703.

15. Ünal İnce E, Ertem M, İleri T, Dalva K, Topcuoğlu P, Uysal Z. Mixed chimerism following hematopoietic stem cell transplantation in pediatric thalassemia major patients: a single center experience. Turk J Hematol. 2010; 27(1): 8-14.
16. Anak S, Sarıbeyoğlu ET. Stem cell transplantation in children. Klinik Gelişim, İstanbul; 2007; pp. 164-76.
17. 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.
18. 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.
19. Friedrich P, Guerra-García P, Stetson A, Duncan C, Lehmann L. Young female donors do not increase the risk of graft-versus-host disease or impact overall outcomes in pediatric HLA-matched sibling hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2018; 24(1): 96-102.
20. Randolph SS, Gooley TA, Warren EH, Appelbaum FR, Riddell SR. Female donors contribute to a selective graft-versus-leukemia effect in male recipients of HLA-matched, related hematopoietic stem cell transplants. Blood. 2004; 103(1): 347-52.
21. Bader P, Kreyenberg H, Hoelle W, Dueckers G, Handgretinger R, Lang P, et al. Increasing mixed chimerism is an important prognostic factor for unfavorable outcome in children with acute lymphoblastic leukemia after allogeneic stem-cell transplantation: possible role for pre-emptive immunotherapy? J Clin Oncol. 2004; 22(9): 1696-705.
22. Delie A, Verlinden A, Beel K, Deeren D, Mazure D, Baron F, et al. Use of chimerism analysis after allogeneic stem cell transplantation: Belgian guidelines and review of the current literature. Acta Clinica Belgica. 2021; 76(6): 500-8.