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The T Cell Receptor Repertoire Diversity Following Hematopoietic Stem Cell Transplantation

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ABSTRACT

Immune reconstitution after hematopoietic stem cell transplantation (HSCT) with a conditioning regimen has appeared to be a promising treatment for autoimmune diseases and hematologic malignancies. This study aimed to assess the T cell receptor (TCR) repertoire diversity in CD4+ cells of patients with hematological malignancies who received allogeneic or autologous HSCT.

The diversity of the TCR repertoire was evaluated in 13 patients with hematologic malignancies before and four months after HSCT. Amino acid changes in the 25 Vβ families were evaluated using Spectratyping and data were presented as Hamming distance (HD). HD more than 20% was considered a change in TCR repertoire after HSCT.

The mean HD was significantly changed after transplantation in all Vβ gene families, with most amino acid changes in p4 and p22 families. There was a strong negative correlation between the HD as the index of TCR repertoire and age (r=−0.62,). The results revealed no association between HD mean and parameters such as sex, disease, conditioning regimen, and type of transplantation.

Our data revealed that commonly used conditioning regimens in Iran could successfully cause TCR repertoire diversity in patients with hematologic malignancies in the short term. The amount of change in TCR repertoire was inversely correlated with the increasing age of patients.

Keywords: Hamming distance; Hematopoietic stem cell transplantation; Spectratyping; T cell receptor

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a therapeutic approach for treating a wide spectrum of blood and immune system disorders, including immunodeficiencies, autoimmune diseases, malignancies, red blood cell disorders, and inherited bone marrow failure syndromes. It starts with the initial depletion of the immunologic memory repertoire, then reconstitution of the immune and hematopoietic systems leads to a comprehensive immunologic regeneration.[2,3] Immune reconstitution (IR) is described by diversifying the repertoire of T cell receptors and functional renewal of regulatory T and B cell compartments and is proposed to reset the immune system.[4-7] Several investigations showed the association of using such therapies with long-term remission in patients.[8,9]. Indeed, one of the crucial factors involved in long-term prognosis after transplantation is IR, especially reconstitution of T cell receptor repertoire, which is vital for disease relapse as well as virus infection.[2] Transplantation factors such as the source of cells, cell dose, and conditioning regimens are important pre-HSCT variables that affect T cell receptor repertoire reconstitution and subsequent complications.[10] Conditioning regimens naturally are myeloablative, and the regimens commonly used for this purpose are total body irradiation (TBI) with cyclophosphamide (Cy) and busulfan (Bu) with Cy.[11,12] Factors like the age of the recipient, previous radiation therapy, comorbidities, issues with TBI delivery, and also the higher occurrence of acute graft-versus-host disease (aGVHD), are the most important factors limiting the use of TBI.[13] In most countries, including Iran, TBI-based conditioning was replaced with high-dose chemotherapy in the majority of HSCT cases administered in order to avoid short- and long-term toxicities related to high-dose TBI.[14,15] The emergence of Bu as an alternative to TBI made it possible for specialists to use TBI-free conditioning regimens with comparable consequences to TBI conditioning.[13]

Nowadays, IR following HSCT with a high-dose chemotherapy conditioning regimen appears to be a promising treatment for autoimmune disease (AD) patients who respond poorly to conventional therapies.[16-18] This improved prognosis is more based on the hypothesis of reconstitution of the TCR repertoire, but due to the lack of sufficient studies, there is still a need for genetic testing. On the other hand, studies indicate, as mentioned above, the conditioning regimen has a significant effect on changing the TCR repertoire and subsequent results. Therefore, considering various conditioning regimens used in Iran and the lack thereof in the Iranian population, there is a need for such an investigation in the country. The present work aimed to evaluate the diversity of TCR in CD4+ cells using the Immunoscope spectrotyping method in patients who received allogeneic or autologous HSCTs.

MATERIALS AND METHODS

Patients

Thirteen patients (10 men and 3 women; mean±SD age, 53±12. years)) with a definitive malignant hematologic disorder diagnosis, who were referred to Imam Khomeini Hospital, Tehran, Iran, for CD34+selected HSCT transplantation were included in the present study. Of these, 3 patients with acute myeloid leukemia (AML) underwent allogeneic CD34+selected HSCT transplantation. The rest of the patients, including 8 patients with multiple myeloma (MM) and 2 with lymphoma, received autologous CD34+selected HSCT transplantation. No intervention in the diagnostic and therapeutic process of patients has been done by the research team. Whole blood samples (15 ml) were taken from patients before and 4 months after transplantation, and also from healthy transplant donors for AML. Three healthy individuals (allogeneic transplant donors for AML patients) were chosen to compare their TCRβ repertoire diversity before and after transplantation. Signed informed consent was taken from all individuals who participated in the present study and all information regard to their medical records were stored on secure system.

Transplant Procedure and Follow-up

The transplantation method and follow-up procedure for patients were based on the routine protocol of Imam Khomeini Hospital, Tehran, Iran, which varied according to the type of malignant hematologic disease.

For AML patients with allogeneic transplantation, busulfan (BU) was administered for 4 consecutive days at 4 mg per kg of body weight every day, and 2 days of Cy at 60 mg/kg/day. Also, on the first day after transplantation, patients were treated with 10 mg/m^2/day of methotrexate (MTX), followed by 15 mg/m^2/day of MTX on the third, sixth, and eleventh day after transplantation.

Multiple myeloma patients,were received Cy at a dose of 60 mg per kg of body weight 15 days before transplantation. Then hematopoietic progenitor cells were mobilized from the bone marrow into the peripheral blood through an infusion of 15 µg/kg granulocyte colony-stimulating factor (G-CSF) in two doses every day (G-CSF*2) for 7 consecutive days from the eighth day before transplantation. On the second day before transplantation, after the last dose of the drug, peripheral blood stem cells were collected. Finally, on the day before transplantation, 100 mg/m2 of Alkeran (an alkylating agent) in two doses with one dose of G-CSF (15 µg/kg/day) was administered. The procedure applied for lymphoma patients was as follows: Patients received Cy at a dose of 60 mg/kg/day, 16 days before transplantation. Then mobilization of hematopoietic progenitor cells from the bone marrow into the peripheral blood was triggered through an infusion of 15 µg/kg of G-CSF in two doses every day (G-CSF*2) for 7 consecutive days from the ninth day before transplantation patients. as with the MM patients, after the last dose of the drug, peripheral blood stem cells were collected (here on the third day before transplantation). Two days before transplantation, 300 mg/m^2/day of VP16, 300 mg/m2/day of Cytarabine, 200 mg/m^2/day of Lomustine, and 15 µg/kg/day of GCSF were administered to patients in one dose. Finally, on the day after (one day before transplantation), patients received 300 mg/m^2/day of VP16, 300 mg/m^2/day of Cy, and 140 mg/m^2/day of Alkeran. Clinical and biological follow-ups were performed daily until the end of aplasia and then followed weekly for 4 months.

Cell Collection

The PBMCs and CD4+ T cells were isolated from whole blood samples collected from patients on the day of transplantation and 4 months after transplantation and also from healthy transplant donors for AML. PBMCs were isolated using the Ficoll method[19] and isolation of CD4+ T cells was performed by the magnetic-activated cell sorting (MACS) method, using the MACS Cell Isolation Kit (Milteny Biotech, Inc.) according to the manufacturer's protocol.

RNA Extraction and cDNA Synthesis

The RNA samples were extracted using RNX-Plus (CinnaGen, Iran) according to the manufacturer's protocol. Briefly, cDNA (1.5 mg) was synthesized using a reverse transcriptase enzyme mix. RT reaction mix (includes oligo (dT) 20 and random hexamers) for one cycle in a final volume of 20 ml was used as follows: 25°C for 10 min, 50°C for 30 min and 85°C for 5 min using Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany). The cDNA was diluted in nuclease-free water and finally stored at −20°C until further use.

Vβ Specific PCR

We used synthesized cDNA in order to amplify 25 separate PCR reactions, each containing one human Vβ family-specific primer that was coupled with one common constant region primer (Supplementary Table).[20] PCR for all primer pairs was performed by mixing 1X PCR buffer, 10 pmol of each unlabeled Vβ specific and constant primers, 0.2 mM dNTP mix, 2.5 U of HotStarTaq DNA Polymerase (Ampliqon, Denmark), and 2.5 µL cDNA in the final reaction volume of 25 µL. Amplification was done on an Eppendorf thermocycler using the following thermal cycling program: 15 minutes at 95°C for initial annealing, followed by 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 90 seconds, and followed by a final extension at 72°C for 10 minutes. The product was used either immediately for run-off PCR or frozen at −20°C until future use.

Primer-extension (Runoff) Reaction

A semi-nested runoff PCR reaction was then performed to label the Vβ-specific PCR product. Runoff PCR reaction mix including 2 µLof Vβ-specific PCR product, 10 pmoL of 4 µM 6-FAM 5′ labeled C-gene runoff primer, 0.2 mM dNTP mix and of 2.5 U of HotStarTaq DNA Polymerase (ampliqon, Denmark) in the final volume of 20 µL was amplified using the following temperature program: 15 min at 95°C for polymerase activation, followed by 30 cycles of 30 sec at 95°C for denaturation, annealing at 60°C for 30 sec, and an extension step at 72°C for 45 sec.

CDR3 Fragment Length Assessment

The ABI 3500 capillary-based electrophoresis genetic analyzer was used to run the labeled PCR product with identical results. Briefly, 1 µL of runoff reaction was mixed with a 9 µL Hi-Di Formamide (4311320 Applied Biosystems, UK) and 0.5 µL of GeneScan™ 500 LIZ Size Standard (cat# 4322682

Applied Biosystems, UK) mixed. The reactions were heated for 5 minutes at 95°C, immediately transferred on ice, and electrophoresed. Osiris software (Version 2.16) was used to read the data. Data obtained from the Osiris software were converted to statistically analyzable data Hamming distance (HD) for reads were calculated. The analyses of HD involved the conversion of the area-under-the-peak data from a spectratype profile into a frequency distribution. When data are presented in the form of frequency distribution, two or more frequency distributions can be compared to each other by summing absolute changes in the frequency of each CDR3 length and expressing the result as a percentage. HD finally determines the difference in TCR repertoire before and after transplantation and scores of more than 20% were considered a change in the repertoire.[21]

Statistical Analysis

Nominal data are reported by number and percentage and quantitative data by mean and standard deviation. The Pearson correlation test was used to examine the correlation between blood indices and HD. The R statistical software (version 4.3) was used to analyze data. p value < 0.05 was considered significant.

RESULTS

Baseline Characteristics

A total of 13 patients (10 men and 3 women) between the ages of 29 and 68 years were included in this study. Demographic and hematological data, including red blood cells (RBCs), white blood cells (WBCs), platelet counts, hemoglobin concentration, lymphocyte, neutrophil, and monocyte percentages before and after transplantation, are shown in Tables 1 and 2 respectively.

TCR Repertoire Profile after HSCT

To evaluate the diversity of the TCR repertoire in 13 individuals who underwent CD34+ selected HSCT, their PBMCs and CD4+ Tcells were collected and analyzed. To assess the degree of similarity between Vβ sequences before and after transplantation, HD was calculated. For each Vβ family, the minimum HD was measured via determination of the minimum number of amino acid differences compared to the same Vβ family before transplantation. By definition, changes of more than 20% were considered significant. As shown in Figure 1, the mean HD was significantly altered after transplantation in all Vβ gene families. According to HD results, most amino acid changes occurred in P4 and P22 Vβ families.

Table 1. Demographics and hematological data from the individuals included in the experiments (before transplantation).

Patient ID	Age (years)	Sex	Disease	RBC	Hb	WBC	Lym (%)	Neut (%)	Monocyte (%)	PLT
1(3)	43	F	AML	3.72	11.7	8.7	44	46	7.60	96
2(7)	53	F	AML	4.7	11.7	8.2	41	41	5	272
3(13)	53	M	AML	3.9	10.6	5.9	26	57	13	370
4(6)	68	M	MM	4.5	14.5	4.9	59	25	13	219
5(8)	56	M	MM	4	12.9	5.7	52	33	11	207
6(10)	62	M	MM	4.7	14.2	3.7	38	39	21	130
7(11)	29	F	Lymphoma	2.8	9	3.8	22	66	9	140
8(12)	35	M	MM	4.9	13.5	4.7	39	55	10	225
9(16)	52	M	MM	5	13.1	2.8	37	52	12	197
10(21)	66	M	MM	5.2	15	7.7	47	40	11	216
11(22)	64	M	MM	4	11.7	8.5	28	66	8	205
12(24)	54	M	MM	5.2	15	6.2	37	55	7	232
13(25)	NA	M	NHL	NA	NA	NA	NA	NA	NA	NA

AML, acute myeloid leukemia; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; RBC, red blood cell; Hb; hemoglobin; WBC, white blood cells; Lym, lymphocyte; Neut, neutrophil; PLT, platelet; M, male; F, female; NA, not available.

Table 2 Demographics and hematological data from the individuals included in the experiments (After transplantation)

Patient ID	Age (year)	Sex	Disease	RBC	HB	WBC	Lym (%)	Neut (%)	Monocyte (%)	PLT
3	43	F	AML	4.3	11.7	5.8	37	54	6	130
7	53	F	AML	3.6	11.7	11.6	38	48	10	361
13	53	M	AML	2.7	10.6	13.8	26	47	5	32
6	68	M	MM	3.6	14.5	4.7	32	57	10	167
7	53	F	AML	3.6	11.7	11.6	38	48	10	361
8	56	M	MM	4	12.9	5.6	39	53	7	185
10	62	M	MM	4	14..2	4.8	42	50	8	125
11	29	F	Lymphoma	2.4	9	3.5	39	52	8	60
12	35	M	MM	4.6	13.5	3.7	44	46	8	183
16	52	M	MM	4.2	13.1	4.6	38	52	8	150
21	66	M	MM	4.3	15	8	27	55	17	279
22	64	M	MM	4.3	11.7	12	9	72	16	362
24	54	M	MM	4.8	15	10	32	58	10	225
25	NA	M	NHL	NA	NA	NA	NA	NA	NA	NA

AML, acute myeloid leukemia; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; RBC, red blood cell; Hb; hemoglobin; WBC, white blood cells; Lym, lymphocyte; Neut, neutrophil; PLT, platelet; M, male; F, female ; NA, not available.

Figure 2 shows the distribution of the mean HD of 25 Vβ families in each of the patients who underwent HSCT after transplantation. Based on our results, the mean change in the TCR repertoire was more than 20% in our patients (except for patient P7).

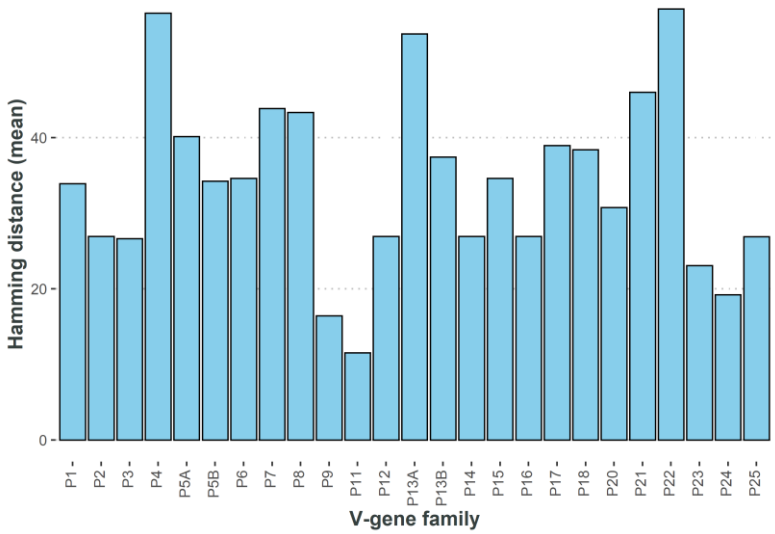

Figure 1. Changes in the TCR V gene family repertoire in hematopoietic stem cell transplant recipients after transplantation

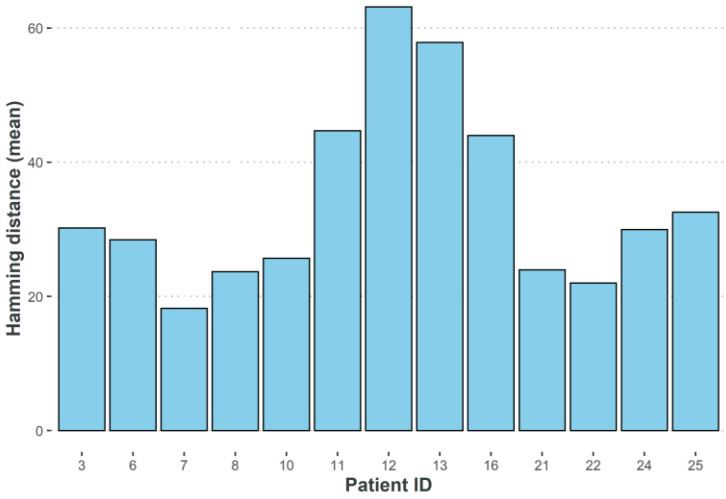

Figure 2. Mean HD distribution of the 25 Vβ families

As shown in Figure 3, there was a strong negative correlation between the index of the TCR repertoire and age (r =−0.62, p = 0.03). We also performed statistical analysis to evaluate the association of effective factors, such as sex, disease, conditioning regimen, and the type of transplantation with the TCR repertoire diversity. We found no association between the HD mean and parameters, such as sex, disease, conditioning regimen, and the type of transplantation (p values of 0.7, 0.8, 0.9, and more than 0.9, respectively).

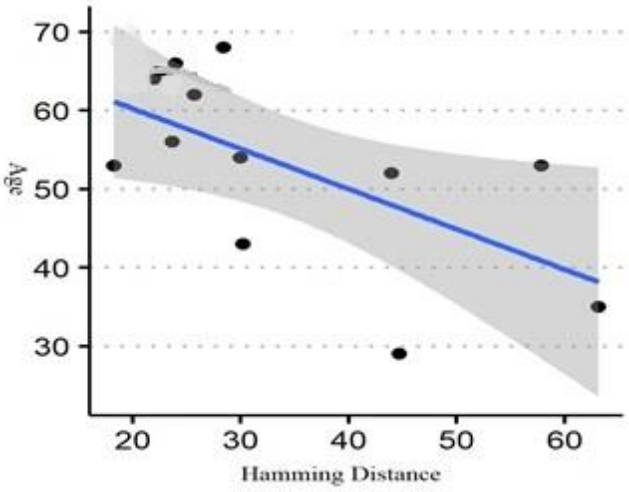

Figure 3. Correlation between the mean hamming distance (HD) as the index of the diversity of TCR repertoire and the age of the patients (n=13).

DISCUSSION

The evaluation of the diversity of the TCR repertoire, which offers a comprehensive image of the distribution and clonal expansion of TCRs, is a useful tool for the characterization of the immune profile of the host T cells and defining the amount of immune reconstitution after HSCT. Also, it could serve as an analysis to evaluate the effect of each HSCT type (autologous or allogeneic) and the conditioning regimens.[2] As a rule, increased diversity of TCR repertoire diversity in transplant recipients represents favorable long-term immune reconstitution after HSCT, which leads to a lower risk of both GVHD and relapse after HSCT transplantation, while delayed or restricted TCR diversity after HSCT is shown to be associated with higher risks of infection and disease relapse following HSCT ([22,23]). The type of HSCT, the conditioning regimen, the composition of the grafts, as well as the occurrence and amount of GVHD are potential contributors that may be associated with delayed TCR repertoire reconstitution after transplantation.[2]

Here, we evaluated the TCR repertoire diversity in patients with hematological malignancies after HSCT. Participants in this study underwent autologous or allogeneic HSCT and received different types of conditioning regimens based on the underlying disease. The results of the present study revealed that regardless of the type of HSCT transplant used and the conditioning regimen administered for the patients, the TCR repertoire in all subfamilies changed significantly after transplantation. The procedure in the present work was performed by spectratyping in order to identify the pattern of CDR3 length distribution and the number of altered amino acids in each of the 25 βV families.[24]

There are two phases of TCR reconstitution after HSCT. The first phase (called thymic independent) involves the homeostatic proliferation of memory T cells, which can be affected by homeostatic cytokines (IL-2, IL-7, and IL-15).[25] This, however, produces a restricted T-cell population with a limited T-cell receptor (TCR) repertoire.[26,27] The second phase is thymus dependent, which is characterized by de novo T-cell development in the thymus.[28] Following TCR rearrangement, newly thymic-generated T cells can undergo stringent selection steps and finally lead to a self-tolerant, highly diverse repertoire of polyfunctional T cells, as supported by the findings in which the seeding of the thymus with newly HSCs lymphoid progenitors in bone marrow recipients.[25]

In 2017, Wiegering by evaluating 182 children who underwent autologous HSCT demonstrated a quicker immune reconstitution in the T-cell compartment, particularly in CD4 and naïve subsets, whereas allogeneic transplant recipients showed a higher proportion of TCRgd.[29] Our investigation showed that there was no difference in the TCR repertoire diversity regarding the type of HSCT. The simple explanation is the small size of our sample, but as they mentioned, participants in their results may be partially affected by the kind of disease. The sample population of the above-mentioned study included both different malignancies and autoimmune diseases, while in the present study only patients suffering from hematological malignancies were included with a significantly higher age range. Otherwise, despite the use of different methodologies, like them, we also observed that eventually, regeneration occurs in all patients.

Farge et al. in two long-term and short-term studies showed that the immune profile of systemic sclerosis, including TCR repertoire, changed dramatically after autologous HSCT.[30,31] Such results were obtained for other autoimmune diseases.[32-34] HSCT protocols were not yet adapted for autoimmune diseases in Iran. However, our results clearly indicate that the conditioning regimens currently used for HSCT candidate patients in Iran can successfully cause changes in the TCR repertoire. These findings promise that clinicians can use these regimens in the near future for patients with autoimmune diseases to achieve immune reconstitution after HSCT. This relies on the hypothesis that after severe depletion of immune cells (autoreactive B and T cells and memory cells and plasma cells) using conditioning regimens, a naïve immune system will regenerate through the hematopoietic stem cells and there will be no more clones to cause autoimmunity. In fact, with HSCT, the immune system will be given a second chance to reconstitute and reset the immune system and this means the cure of autoimmune diseases.[35,36]

Previous studies have investigated the effect of various types of conditioning regimens on HSCT outcomes.[10,14] The conditioning protocol applied for HSCT in Iran is different from other HSCT centers in the world by using a TBI-free conditioning regimen for all therapeutic indications of HSCT treatment.[37] Our

data showed that this conditioning regimen was successful in the induction of immune reconstitution, which was in line with the results of research performed by Chen and colleagues.[38] In 2006, they evaluated the effect of reduced-intensity conditioning (RIC) protocol compared to the TBI and ATG regimens in 22 patients with refractory hematological malignancies. They showed that compared to protocols that used myeloablative regimens, these patients showed faster recovery in TCD3+ cells and an increase in the diversity of the TCRβ repertoire.[38]

Our analysis revealed that the TCR repertoire diversity had a strong negative correlation with the increasing age of the participants. As we know, the thymus becomes atrophic and loses its function as the age of individuals increases.[39] The association between delayed T-cell immune reconstitution and increased age has previously been demonstrated.[40] In line with our study, Britanova et al. revealed a strong inverse correlation between increasing age and TCR repertoire diversity.[41]

Despite the introduction of novel methods such as next generation sequencing and microarray, the spectratyping technique is considered the gold standard method for TCR repertoire evaluation. In addition to the validity of its results, this method is a cost-effective and feasible test that can be set up in any laboratory.[42] In the future, a comprehensive investigation with a larger sample size could lead to more reliable and precise results regarding the effect of using a particular conditioning regimen on the TCR repertoire diversity after HSCT.

However, we acknowledge the limitations of our study, which includes the lack of autoimmune patients who were candidates for transplantation and the self withdrawal of some patients to further participate in this study at 4 months post transplantation.

In conclusion, here we showed that those conditioning regimens that are commonly used for both types of HSCT transplantation (autologous and allogeneic) in Iran, in the short term, successfully cause TCR repertoire diversity in patients with hematologic malignancies. The amount of change in the TCR repertoire was inversely correlated with the increasing age of the patients. Since it is not common to perform HSCT in autoimmune diseases in Iran, the successful change of TCR repertoire in the present study could be a promise that in the future, clinicians can consider HSCT as an option for patients with these diseases.

STATEMENT OF ETHICS

The study was approved by the ethical committee of the Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.MSP.REC.1396.474).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

1. Epah J, Schafer R. Implications of hematopoietic stem cells heterogeneity for gene therapies. Gene Ther. 2021;28(9):528-41.
2. Li Y, Xu L. Evaluation of TCR repertoire diversity in patients after hematopoietic stem cell transplantation. Stem Cell Investig. 2015;2:17.
3. Swart JF, Delemarre EM, van Wijk F, Boelens JJ, Kuball J, van Laar JM, et al. Haematopoietic stem cell transplantation for autoimmune diseases. Nat Rev Rheumatol. 2017;13(4):244-56.
4. Abrahamsson S, Muraro PA. Immune re-education following autologous hematopoietic stem cell transplantation. Autoimmunity. 2008;41(8):577-84.

5. Arruda LCM, Malmegrim KCR, Lima-Junior JR, Clave E, Dias JBE, Moraes DA, et al. Immune rebound associates with a favorable clinical response to autologous HSCT in systemic sclerosis patients. Blood Adv. 2018;2(2):126-41.
6. Delemarre EM, van den Broek T, Mijnheer G, Meerding J, Wehrens EJ, Olek S, et al. Autologous stem cell transplantation aids autoimmune patients by functional renewal and TCR diversification of regulatory T cells. Blood. 2016;127(1):91-101.
7. Muraro PA, Douek DC, Packer A, Chung K, Guenaga FJ, Cassiani-Ingoni R, et al. Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. J Exp Med. 2005;201(5):805-16.
8. Kalincik T, Brown JWL, Robertson N, Willis M, Scolding N, Rice CM, et al. Treatment effectiveness of alemtuzumab compared with natalizumab, fingolimod, and interferon beta in relapsing-remitting multiple sclerosis: a cohort study. Lancet Neurol. 2017;16(4):271-81.
9. Mancardi G, Sormani MP, Muraro PA, Boffa G, Saccardi R. Intense immunosuppression followed by autologous haematopoietic stem cell transplantation as a therapeutic strategy in aggressive forms of multiple sclerosis. Mult Scler. 2018;24(3):245-55.
10. de Koning C, Nierkens S, Boelens JJ. Strategies before, during, and after hematopoietic cell transplantation to improve T-cell immune reconstitution. Blood. 2016;128(23):2607-15.
11. Park H, Byun JM, Koh Y, Yoon SS, Park H, Lee J, et al. Comparison of Different Conditioning Regimens in Allogeneic Hematopoietic Stem-Cell Transplantation Shows Superiority of Total Body Irradiation-Based Regimen for Younger Patients With Acute Leukemia: A Nationwide Study. Clin Lymphoma Myeloma Leuk. 2019;19(11):e605-e15.
12. Storb R. History of pediatric stem cell transplantation. Pediatr Transplant. 2004;8 Suppl 5:5-11.
13. Jethava YS, Sica S, Savani B, Socola F, Jagasia M, Mohty M, et al. Conditioning regimens for allogeneic hematopoietic stem cell transplants in acute myeloid leukemia. Bone Marrow Transplant. 2017;52(11):1504-11.
14. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. Blood. 2014;124(3):344-53.
15. Hunger SP, Loh KM, Baker KS, Schultz KR. Controversies of and unique issues in hematopoietic cell transplantation for infant leukemia. Biol Blood Marrow Transplant. 2009;15(1 Suppl):79-83.
16. Atkins HL, Bowman M, Allan D, Anstee G, Arnold DL, Bar-Or A, et al. Immunoablation and autologous haemopoietic stem-cell transplantation for aggressive multiple sclerosis: a multicentre single-group phase 2 trial. Lancet. 2016;388(10044):576-85.
17. Passweg JR, Baldomero H, Bader P, Basak GW, Bonini C, Duarte R, et al. Is the use of unrelated donor transplantation leveling off in Europe? The 2016 European Society for Blood and Marrow Transplant activity survey report. Bone Marrow Transplant. 2018;53(9):1139-48.
18. Snowden JA, Badoglio M, Labopin M, Giebel S, McGrath E, Marjanovic Z, et al. Evolution, trends, outcomes, and economics of hematopoietic stem cell transplantation in severe autoimmune diseases. Blood Adv. 2017;1(27):2742-55.
19. Seif F, Khoshmirsafa M, Mousavi M, Beshkar P, Rafeian-Kopaei M, Bagheri N, et al. Interleukin-21 receptor might be a novel therapeutic target for the treatment of rheumatoid arthritis. Journal of Experimental & Clinical Medicine. 2014;6(2):57-61.
20. Currier JR, Deulofeut H, Barren KS, Kehn PJ, Robinson MA. Mitogens, superantigens, and nominal antigens elicit distinctive patterns of TCRB CDR3 diversity. Human immunology. 1996;48(1-2):39-51.
21. Currier JR, Robinson MA. Spectratype/immunoscope analysis of the expressed TCR repertoire. Current protocols in immunology. 2000;38(1):10.28. 1-10.28. 4.
22. Toubai T, Hirate D, Shono Y, Ota S, Ibata M, Mashiko S, et al. Chimerism and T-cell receptor repertoire analysis after unrelated cord blood transplantation with a reduced-intensity conditioning regimen following autologous stem cell transplantation for multiple myeloma. Int J Lab Hematol. 2008;30(1):75-81.
23. Yew PY, Alachkar H, Yamaguchi R, Kiyotani K, Fang H, Yap KL, et al. Quantitative characterization of T-cell repertoire in allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant. 2015;50(9):1227-34.
24. Fozza C, Barraqueddu F, Corda G, Contini S, Virdis P, Dore F, et al. Study of the T-cell receptor repertoire by CDR3 spectratyping. J Immunol Methods. 2017;440:1-11.
25. Gaballa A, Clave E, Uhlin M, Toubert A, Arruda LC. Evaluating thymic function after human hematopoietic stem cell transplantation in the personalized medicine era. Frontiers in Immunology. 2020;11:1341.
26. Danby R, Rocha V. Improving engraftment and immune reconstitution in umbilical cord blood transplantation. Front Immunol. 2014;5:68.

27. Seggewiss R, Einsele H. Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. Blood. 2010;115(19):3861-8.
28. van Den Brink M, Leen AM, Baird K, Merchant M, Mackall C, Bollard CM. Enhancing immune reconstitution: from bench to bedside. Biol Blood Marrow Transplant. 2013;19(1 Suppl):S79-83.
29. Wiegering V, Eyrich M, Winkler B, Schlegel PG. Comparison of immune reconstitution after allogeneic vs. autologous stem cell transplantation in 182 pediatric recipients. Pediatric Hematology Oncology Journal. 2017;2(1):2-6.
30. Farge D, Arruda LCM, Brigant F, Clave E, Douay C, Marjanovic Z, et al. Long-term immune reconstitution and T cell repertoire analysis after autologous hematopoietic stem cell transplantation in systemic sclerosis patients. Journal of Hematology & Oncology. 2017;10(1):21.
31. Farge D, Henegar C, Carmagnat M, Daneshpouy M, Marjanovic Z, Rabian C, et al. Analysis of immune reconstitution after autologous bone marrow transplantation in systemic sclerosis. Arthritis Rheum. 2005;52(5):1555-63.
32. Pockley AG, Lindsay JO, Foulds GA, Rutella S, Gribben JG, Alexander T, et al. Immune Reconstitution After Autologous Hematopoietic Stem Cell Transplantation in Crohn's Disease: Current Status and Future Directions. A Review on Behalf of the EBMT Autoimmune Diseases Working Party and the Autologous Stem Cell Transplantation In Refractory CD-Low Intensity Therapy Evaluation Study Investigators. Front Immunol. 2018;9:646.
33. Ruder J, Rex J, Obahor S, Docampo MJ, Muller AMS, Schanz U, et al. NK Cells and Innate-Like T Cells After Autologous Hematopoietic Stem Cell Transplantation in Multiple Sclerosis. Front Immunol. 2021;12:794077.
34. Szodoray P, Varoczy L, Papp G, Barath S, Nakken B, Szegedi G, et al. Immunological reconstitution after autologous stem cell transplantation in patients with refractory systemic autoimmune diseases. Scand J Rheumatol. 2012;41(2):110-5.
35. Arruda LC, Clave E, Moins-Teisserenc H, Douay C, Farge D, Toubert A. Resetting the immune response after autologous hematopoietic stem cell transplantation for autoimmune diseases. Curr Res Transl Med. 2016;64(2):107-13.
36. Lutter L, Spierings J, van Rhijn-Brouwer FCC, van Laar JM, van Wijk F. Resetting the T Cell Compartment in Autoimmune Diseases With Autologous Hematopoietic Stem Cell Transplantation: An Update. Front Immunol. 2018;9:767.
37. Hamidieh AA, Behfar M, Babaki AE, Jalali A, Hosseini AS, Jahani M, et al. Hematopoietic SCT in Iranian children 1991-2012. Bone Marrow Transplant. 2015;50(4):517-22.
38. Chen X, Hale GA, Barfield R, Benaim E, Leung WH, Knowles J, et al. Rapid immune reconstitution after a reduced-intensity conditioning regimen and a CD3-depleted haploidentical stem cell graft for paediatric refractory haematological malignancies. Br J Haematol. 2006;135(4):524-32.
39. Hakim FT, Memon SA, Cepeda R, Jones EC, Chow CK, Kasten-Sportes C, et al. Age-dependent incidence, time course, and consequences of thymic renewal in adults. J Clin Invest. 2005;115(4):930-9.
40. Lin RJ, Elias HK, van den Brink MRM. Immune Reconstitution in the Aging Host: Opportunities for Mechanism-Based Therapy in Allogeneic Hematopoietic Cell Transplantation. Front Immunol. 2021;12:674093.
41. Britanova OV, Putintseva EV, Shugay M, Merzlyak EM, Turchaninova MA, Staroverov DB, et al. Age-related decrease in TCR repertoire diversity measured with deep and normalized sequence profiling. J Immunol. 2014;192(6):2689-98.
42. Gkazi AS, Margetts BK, Attenborough T, Mhaldien L, Standing JF, Oakes T, et al. Clinical T Cell Receptor Repertoire Deep Sequencing and Analysis: An Application to Monitor Immune Reconstitution Following Cord Blood Transplantation. Front Immunol. 2018;9:2547.