

Original Article**Effect of Chimerism on Relapse in Stem Cell Transplantation****Ahmet Kaya¹, Mehmet Ali Erkurt^{1*}, İrfan Kuku¹, Emin Kaya¹, İlhami Berber¹, Ahmet Sarıcı¹, Süleyman Arslan¹, Soykan Biçim², Fatma Hilal Yagın³**¹Department of Hematology, Turgut Ozal Medical Center, Inonu University, Malatya, Turkey.²Department of Hematology, Malatya Education and Research Hospital, Malatya, Turkey.³Department of Biostatistics and Medical Informatics, Inonu University, Faculty of Medicine, Malatya, Turkey.**Abstract**

Background: In this study, the relationship between the rate of chimerism and disease recurrence after stem cell transplantation was examined.

Methods: Chimerism results, stem cell transplantation between 5.10.2018 and 21.12.2022, were evaluated. According to the most recently measured chimerism value, patients were divided into groups as full chimerism (>95%), mixed chimerism between 95% and 5%, and non-chimerism below 5%. The association of full chimerism, mixed chimerism and non-chimerism groups with relapse was examined. The recurrence status of the patients was determined by clinical, flow cytometry and the most important parameter, bone marrow examination and pathology reports. How many days after the transplant patients relapsed and whether the recurrence was related to the chimerism status was examined.

Results: Statistically significant correlation was found between chimerism and relapse ($p=0.006$). Results showed that patients with mixed chimerism were more likely to relapse. There was a statistically significant difference between the chimerism groups in terms of time to relapse ($p=0.013$) and time to deceased ($p=0.028$). Relapse and time to death were significantly lower in patients with full chimerism compared to patients with non-chimerism and mixed chimerism.

Conclusion: There is a relationship between chimerism and relapse in stem cell transplantation.

Keywords: Stem cell transplantation, Chimerism, Relaps.

Introduction

Chimerism guides the clinician about the patient's immune response after stem cell transplantation. Chimerism is studied in which unique DNA fragments in both the donor and recipient are used as markers using stem cell pre-transplant DNA samples. Post-transplantation, the recipient is evaluated for these uniquely identifying DNA fragments. In the absence of donor DNA fragments detected in recipient samples, graft rejection can be considered. Ultimately the recipient DNA fragments are a proof of the recipient's hematopoiesis (1). Chimerism can be studied in lymphocyte and granulocyte serial elements (T, B, NK and CD34) from peripheral blood or bone marrow (2). Chimerism may have an sign on potential graft rejection and early recurrence, as well as affect the patient's post-transplant treatment decisions (3). Various methods can be used to assess chimerism after stem cell transplantation, including quantitative fluorescent polymerase chain reaction, quantitative real-time detection of short tandem repeats of single nucleotide polymorphisms. In the case of sex-inconsistent transplants, the fluorescent in situ hybridization method for the Y chromosome can be used (4).

Chimerism types and terminology: Chimerism is a condition that refers to the detection of donor cells in the recipient after allogeneic stem cell transplantation (5). Chimerism can be shown as mixed and full chimerism. Mixed chimerism is the presence of donor and recipient cells in varying proportions in the recipient blood. In full chimerism, the recipient blood is dominated by donor cells. Chimerism can also be expressed as permanent and temporary. In permanent chimerism, donor cells can be detected for a long time after transplantation, while in temporary chimerism it is the inability to detect the genetic material of the donor within weeks after the transplant. While microchimerism refers to cell material in which donor cell materials can be detected with sensitive methods, macrochimerism means that there are hematopoietic cells from many lineages of donor origin and the dominant cell source is donor origin (6). Chimerism is usually defined as a percentage. Acceptable levels of chimerism are assessed based on the underlying condition (inherited, acquired benign versus malignant), chemotherapy regimen used at the time of transplantation (myeloablative versus low-intensity non-myeloablative priming regimens), study timing, clinical situation (7). Allogeneic transplant recipients are

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screened periodically with chimerism studies that identify the origin of post-transplant hematopoiesis. Hematopoiesis is composed of more than 95% donor cells in complete chimerism consisting entirely of donor, a value over 5% is encountered in mixed chimerism involving different ratio of donor and recipient cells, or a result of chimerism below 5% in case of graft rejection (consisting entirely of recipient cells) (7).

Chimerism mechanism of action: Chimerism is thought to reinforce central immune tolerance by clonal selection of recipient reactive T cells. Settlement of donor dendritic cells into the recipient thymus provides clonal evolution to donor reactive T cells and promotes clonal deletion of recipient reactive T cells (8). Peripheral mechanisms other than central immune tolerance have also been identified. An increase in donor-specific regulatory T cells (Treg) has been shown in recipients that are tolerant to donor cells. Tregs are thought to be functional outside the central immune system (9).

Chimerism analysis: Chimerism in allogeneic stem cell transplantation has an important place in transplantation monitoring. Historically, cytogenetic analyzes such as erythrocyte phenotyping and chromosome analysis or fluorescent in situ hybridization have been used for chimerism analysis (10). DNA-based techniques such as fragment polymorphisms, variable number of tandem repeats, and short tandem repeats (STR) have been used as safe methods (11). Today, PCR-assisted analysis of STRs is most frequently used. STR loci are polymorphic in humans, making them definitive targets (12). Real-time quantitative PCR (qPCR), digital droplet PCR (dPCR) and lastly next generation sequencing (NGS) are the most up-to-date and reliable techniques that many laboratories cannot reach (13). In order to study chimerism after transplantation, donor and pre-transplant recipient samples are needed. STR analysis method is the most frequently used method in chimerism studies. STR analysis provides information in the evaluation of donor and recipient compatibility (rejection, recurrence, acceptance) in stem cell transplantation. Each STR locus has two alleles (inherited from parents). The STR locus can be homozygous or heterozygous. Each STR locus focus is classified as informative, non-informative, and partially informative focus. For the focus to be informative, at least one allele must be unique in the donor and recipient. It is not informative when the genetic locus is shared between recipient and donor. However, unique loci in the recipient are considered partially informative and are a factor that makes chimerism calculations difficult. These loci are not recommended in chimerism testing. The informative STR locus naturally decreases as the kinship relationship between the donor and recipient increases (14).

Chimerism /relapse: Chimerism is the best predictor of stem cell transplant outcome in malignant hematological diseases. By using chimerism, one can get an idea about the course of the disease and relapse after transplantation. The early decrease in donor chimerism after transplantation is considered a risk factor for relapse. Chimerism <90% within 100 days after transplantation increases relapse and reduces overall survival (15). As in malignant hematological diseases, mixed chimerism has been associated with relapse in benign hematological diseases. It has been observed that in benign hematological diseases, there is an increase in the frequency of relapse in parallel with the level of mixed chimerism and overall survival is affected (16).

The predictability of chimerism in graft rejection after hematopoietic stem cell transplantation is a matter of debate. In this study, we examined the effect of chimerism status on graft rejection after allogeneic stem cell transplantation.

Materials and Methods

Ethical status: The study was started after the decision number 19- 2022/4071 dated 29.11.2022 was approved on İnönü university scientific research and publication ethics committee (health sciences non-interventional clinical research ethics committee). A total of 148 patients, 49 female and 99 male, were included in the study. The patients included in the study were those over the age of 18 who received adult allogeneic stem cell transplantation. All patients included in the study complied with the Helsinki code of ethics for medical research.

Participation confirmation statement: Informed consent forms of the patients are available in the patient file. Patient files are kept in the adult bone marrow transplant unit archive.

Data Availability Statement: The study data are available in the hospital electronic registration system and the genetics laboratory electronic registration system, but are not publicly available for ethical reasons. If necessary, they can be obtained from the corresponding author by fulfilling legal responsibilities.

Study design: Chimerism results, which were checked periodically at 1,2,3,6,9,12,18,24,36,48 months in patients who had stem cell transplantation between 5.10.2018 and 21.12.2022, were evaluated.

According to the most recently measured chimerism value, patients were divided into groups as full chimerism (>95%), mixed chimerism between 95% and 5%, and non-chimerism below 5%.

The association of full chimerism, mixed chimerism and non-chimerism groups with relapse was examined. The recurrence status of the patients was determined by clinical, flow cytometry and the most important parameter, bone marrow examination and pathology reports.

How many days after the transplant patients relapsed and whether the recurrence was related to the chimerism status was examined.

Study material information: Chimerism studies of the patients were studied with mentype DIPscreen Biotype Dresden/GERMANY Real time-polymerase chain reaction kits and analyzed with the 3500 Genetic Analyzer 8ch RUO model(622) part no (0010) HITACHI-To-kyo/JAPAN device.

Data analysis: Qualitative data were summarized as number-percent-age. Relationships between qualitative data were evaluated with the Pearson Chi-Square test. The distribution of quantitative variables was determined by the Shapiro-Wilk test. Quantitative variables were represented by median and interquartile range (IQR). Differences between chimerism groups were examined by the Kruskal-Wallis test. Statistical values with a p value below 5% were considered significant. All statistical analyzes were performed in IBM SPSS Statistics for Windows version 28.0 (New York; USA).

Results

A total of 148 patients, 49 (33.1%) female and 99 (66.9%) male, were included in the study. The mean age of the patients was 38±14, and recurrence was observed in 39 (26.4%) patients, while recurrence was not observed in 109 (73.6%) patients. 49 (33.1%) of the patients were deceased and relapse was observed in 29 (59.1%) of these patients, while 20 (40.8%) did not have recurrence. 37 (30%) of 123 full chimeric patients, 9 (47.3%) of mixed chimeric 19 patients and 3 (50%) of 6 non-chimeric patients were found to be deceased. 6 (4.1%) patients did not have chimerism, 19 (12.8%) had mixed chimerism, 123 (83.1%) had full chimerism. Descriptive statistics of patients according to chi-

Table 1: Descriptive statistics of patients by chimerism groups.

Variable		CHIMERISM		
		non- chimerism	mix chimerism	full chimerism
		n(%)	n(%)	n(%)
GENDER	FEMALE	2 (33.33)	5 (26.32)	42 (34.15)
	MALE	4 (66.67)	14 (73.68)	81 (65.85)
DIAGNOSIS	AA	0 (0.00)	1 (5.26)	12 (9.76)
	ALL	0 (0.00)	7 (36.84)	29 (23.58)
	AML	4 (66.67)	10 (52.63)	62(50.41)
	HL	0 (0.00)	0 (0.00)	9 (7,31)
	CLL	0 (0.00)	0 (0.00)	1 (0.81)
	CML	0 (0.00)	0 (0.00)	3 (2.44)
	MDS	0 (0.00)	0 (0.00)	2 (1.63)
	MYELOFIBROSIS	1 (16.67)	0 (0.00)	1 (0.81)
	PNH	0 (0.00)	1 (5.26)	1 (0.81)
	THALASSEMIA	1 (16.67)	0 (0.00)	3 (2.44)
TYPE OF TRANSPORT	NON KINSHIP MATCH (8/8)	0 (0.00)	4 (21.05)	24 (19.51)
	HAPLIDENTIC MISMATCH (≤6/8)	0 (0.00)	1 (5.26)	0 (0.00)
	KINSHIP MATCH (8/8) or MISMATCH (7/8)	6 (100.00)	14 (73.68)	99 (80.49)

Abbreviations: AA-aplastic anemia, ALL- acute lymphocytic leukemia, AML-, acute myelocytic leukemia, HL- hodgin lymphoma, CLL-chronic lymphocytic leukemia, CML-chronic myelocytic leukemia, MDS-myelodysplastic syndrome, PNH- paroxismal nocturnal hemoglobinuria

Table 2: Statistics on the associations between chimerism and relapse

Variable		CHIMERISM			p-value
		non- chimerism	mix chimerism	full chimerism	
		n(%)	n(%)	n(%)	
RELAPS	RELAPS	3 (50.00)	10 (52.63)	26 (21.14)	0.006*
	NO RELAPS	3 (50.00)	9 (47.37)	97 (78.86)	

*: Statistical significance.

Table 3: Statistics on the relationship between Chimerism and relapse and deceased day in patients with relapse

Variable	CHIMERISM			p-value
	Non chimerism	mix chimerism	full chimerism	
	Median (IQR)	Median (IQR)	Median (IQR)	
AFTER TRANSPLANT RELAPSE (DAYS)	399(211)	229.5(504)	119.5(79) _a	0.013*
AFTER TRANSPLANT DECEASED	529(97)	212(494)	133(25) _a	0.028*

a: There is a statistically significant difference between non-chimerism and mixed chimerism groups (p<0.05);

*: Statistical significance.

merism groups are given in Table 1.

Statistically significant correlation was found between chimerism and recurrence . It was observed that recurrence was more common in patients with mixed chimerism (p=0.006, Table 2).

When the effect of the chimerism groups on the time of recurrence and the time of death of the patient was examined in relapsed patients, there was a statistically significant difference between the chimerism groups in terms of time to relapse (p=0.013) and time to death (p=0.028). Both time to recurrence and time to death were significantly lower in patients with full chimerism compared to patients with non-chimerism and mixed chimerism (Table 3).

When the chimerism status is examined, there is a statistically significant difference in terms of chimerism rate. According to post-hoc analysis, the rate of chimerism was significantly higher in the full chimerism group compared to the non-chimerism and mixed chimerism

groups , however, there was no difference in the rate of chimerism between non-chimerism and mixed chimerism groups. In addition, there was no statistically significant difference between the chimerism groups in terms of the day the chimerism was studied after the transplant (Table 4).

Discussions

In the study by Mellgren et al., early analysis of chimerism in peripheral blood can be used to identify patients at high risk of graft rejection, however, early chimerism analysis has limited effectiveness in predicting leukemia relapse(17).

In this study, as a result of the latest chimerism analysis of patients from peripheral blood, non-mix and full chimerism recurred at a rate of 50, 52.63 and 21.14%, respectively (p 0,006). Patients with non-mix full chimeric relapse after stem cell transplantation, on average 269, 126, and 170 days, respectively. It is observed that patients with mixed

chimeric experience an earlier recurrence compared to other groups. The high recurrence rate in the non and mixed chimeric group may be related to the lower number of patients.

In the study of Qin et al., Twenty-one patients experienced a leukemia recurrence at a median of 135 days after transplantation. All patients had elevated recipient chimerism in their bone marrow sample at the time of relapse, and 90% of patients had increased recipient chimerism in their bone marrow sample prior to relapse (18).

In the analyzes of this study, recurrence was observed in patients with non-mix full chimerism after transplantation on a mean of 399, 229, and 119 days, respectively. Non-mix and full chimerism ratios were calculated as 2.3, 92.6, and 99.3 on average, respectively. non-chimeric 50%, mixed chimeric 52.63% full chimeric 21.14% of the patients had recurrence. The increase in the recipient specific DNA ratio in patients is not parallel to the recurrence. Not every patient with low chimerism relapses, and full chimerism could not be evaluated as in remission in every patient. This situation in transplantation indicates that there are different immune mechanisms in transplantation, as it is due to the fact that the insufficient number of our patients affects the statistics.

In a study of 100 patients undergoing allogeneic transplantation for acute myelocytic leukemia, Bouvier et al., In patients without full chimerism, defined as less than 0.01% of recipient DNA in CD3-negative cells, was associated with a significantly higher risk of recurrence and a lower overall survival (19).

Chimerism studies are not screened for CD3 in patients undergoing stem cell transplantation in our institution. Chimerism studied from a peripheral blood sample is assumed to be DNA of granulocytes and lymphocytic cells. In this study, 21.14% of full chimeric patients had recurrence after an average of 119.5 days. Although survival analysis was not performed in our study, it was seen that 33.1% of the patients died and 59.1% of the patients with this deceased had recurrence. full chimeric 30%, mixed chimeric 47.3% non chimeric 50% deceased. Although the data of this study are in parallel with the literature, the number of non and mixed chimeric patients is very low and it is thought that this situation may affect the statistical analysis.

Conclusion

There is a relationship between chimerism and recurrence in stem cell transplantation. The fact that this relationship is inversely proportional to the rate of chimerism was not supported by the data of this study. In order to elucidate the relationship between chimerism and disease recurrence, studies with more non- and mixed chimeric patient groups are needed.

Conflicts of Interest: The authors declare no conflicts of interest.

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