# Evaluation of Clinical Outcome and Mixed Chimerism Follow-up after Allogeneic Hematopoietic Stem Cell Transplantation in Hematological Malignancies

## Hematolojik Malignitelerde Allojenik Hematopoetik Kök Hücre Transplantasyonu Sonrası Miks Kimerizmin Takibi ve Kliniğe Yansımalarının Değerlendirilmesi

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**ABSTRACT**

**Objective:** The level of donor-recipient chimerism is an established method to document donor engraftment. Allogeneic stem cell transplantation(ASCT) may result in mixed hematopoietic chimerism (MC), especially after reduced intensity conditioning regimens. Increasing MC levels in the post ASCT period may indicate disease relapse, graft failure, or rejection. In this study we aimed to study clinical outcome of mixed chimerism after ASCT in patients with hematological diseases.
**Methods:** The data of 335 patients whose ASCT were performed at our center between 2009 and 2019 and survived more than three months after transplantation were analyzed retrospectively.
**Results:** During follow up period 127 (43%) of 293 patients in full chimeric (FC) group and 11 (26.1%) of 42 patients in MC group died. 11 patients received donor lymphocyte infusions (DLI) after MC detected, 4 of them converted into FC. 10 mix chimeric patients converted into FC spontaneously. In 3 patients no donor cell was observed in time (0%). 2 of them are still in remission. 25 patients remained mix chimeric during follow up period. 66 patients received reduced intensity conditioning (RIC) regimen and 10 (15.1%) of them had MC during follow up period. 269 patients received myeloablative conditioning (MAC) regimen and 32 (11.8%) of them had MC during follow up period.
**Conclusion:** ASCT may result in MC especially after RIC regimens. MC does not always related with relapse risk and mix chimeric patients with malignant or benign hematological diseases can stay in remission for a long time.

**Key Words:** Chimerism, stem cell transplantation, allogeneic

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**ÖZET**

**Amaç:** Alıcının kimerizm düzeyinin takibi, donör engraftrasyonunu tespit etmek için kullanılan bir yöntemdir. Allojeneik kök hücre nakli (AKHN), özellikle düşük yoğunluklu hazırlıktan sonra karışık tip kimerizme neden olabilir. AKHN sonrası karışık tip kimerizm seviyelerinin artması, hastalık nüksünü, greft yetmezliğini veya reddini gösterebilir. Bu çalışmada, hematolojik malignite sebebiyle AKHN yapılan hastalarda, nakil sonrası karışık tip kimerizmin kliniğe yansımaları değerlendirildi.
**Yöntem:** Merkezimizde 2009-2019 yılları arasında AKHN uygulanan ve transplantasyon sonrası sağkalımı üç ayın üzerinde olan, 335 hastanın verileri retrospektif olarak incelendi.
**Bulgular:** Takipler sırasında, tam kimerik gruptan 293 hastanın 127'si (%43) ve karışık tip kimerizm grubundan ise 42 hastanın 11'i (%26.1) kaybedildi. Karışık tip kimerizm saptanan 11 hastaya donör lenfosit infüzyonu uygulandı, işlem sonrası 4 hasta tam kimerizme döndü. Yine karışık tip kimerizm tespit edilen 10 hasta ise kendiliğinden tam kimerizme dönmüştür. Takipler sırasında üç hastada donöre ait hücre gözlenmedi (% 0). Bu hastaların ikisi hala remisyonda takip edilmektedir. Karışık tip kimerizmde kalan hasta sayısı 25'tir. Düşük yoğunluklu hazırlık 66 hastaya uygulandı ve bunların 10'unda (%15.1) karışık tip kimerizm tespit edildi. 269 hastaya miyeloablatif kondisyon rejimi uygulandı ve bunların 32'sinin (%11.8) takiplerinde karışık tip kimerizm tespit edildi.
**Sonuç:** AKHN, özellikle düşük yoğunluklu hazırlıktan sonra karışık tip kimerizm ile sonuçlanabilir. Karışık tip kimerizm her zaman nüks riskiyle ilişkili değildir ve bu durumda uzun süre remisyonda kalabilirler.

**Anahtar Sözcükler:** Kimerizm, kök hücre nakli, allojenik

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## INTRODUCTION

Allogeneic stem cell transplantation (ASCT) is a potentially curative treatment used in a variety of benign and malignant hematologic diseases, solid tumors, neurological disorders autoimmune diseases and immunodeficiencies. Early after ASCT, infections and acute graft-versus-host disease (GVHD) are major causes of death (1). The outcome of ASCT in malignant hematological diseases depends mainly on the immunological graft-versus-leukemia effect of donor cell reactivity against host malignant cells wheras in benign hematological diseases it depends mainly on the replacement of donor's cells instead of recipient's cells and recovery of hematopoiesis.

Conditioning regimens for ASCT have variable intensity, toxicity and have been termed as myeloablative, reduced intensity, and non myeloablative regimens. Myeloablative conditioning (MAC) regimen results in a long-lasting pancytopenia that is irreversible unless hematopoiesis is restored by infusion of donor stem cells. A non myeloablative (NMA) regimen cause minimal cytopenia and may not require stem cell support. Reduced intensity conditioning (RIC) regimens cause cytopenias, which may be prolonged and require hematopoietic stem cell support (2-5). RIC regimens could decrease toxicities related to ASCT therefore, RIC has extended the approach of ASCT in patients who are not eligible candidates for standard ASCT because of their advanced age and/or comorbidities (6-9). 100 days after ASCT, relapse is the major cause of death and its incidence and outcome have not significantly improved over the last decades (1). A randomized study compared RIC and MAC conditioning regimens and found that RIC was associated with more relapse (10). The risk of relapse after RIC is 25–60% (11–16), and the median time to disease relapse is 3–7 months (17–20), therefore evaluating patients to detect relapse early in the post-transplant period is very important especially in patients who received NMA or RIC regimens. If relaps can be detected early after ASCT, treatments that can potentially prevent disease recurrence and improve survival such as maintenance regimens or donor lymphocyte infusions (DLI) should be considered (21).

The level of donor-recipient chimerism is an established method to document donor engraftment (22). ASCT may result in mixed hematopoietic chimerism (MC), especially after RIC regimens and after T-cell depletion (23,24). Increasing MC levels in the post ASCT period may indicate disease relapse, graft failure, or rejection. On the other hand, decreasing MC, often seen after tapering of immunosuppression after transplant or after DLI, may be an early predictor of GvHD or graft-versus-tumor effect (25). In this study we aimed to study clinical outcome of mixed chimerism after ASCT in patients with hematological diseases.

## MATERIALS and METHOD

*Sample Preparation*

In this study, the data of ≥18 years old patients whose ASCT were performed at Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Bone Marrow Transplantation Center between 2009-2019 were analyzed retrospectively. Patients died due to transplant related mortality in the first 100 days after ASCT were excluded from the study.

Disease relapse was defined as morphologic, cytogenetic or radiologic evidence of disease demonstrating pre-transplant characteristics. Bone marrow biopsies were routinely performed at day 30. Donor-recipient chimerism levels were measured in the peripheral blood on day 30, 60, 90, and then every 3 months in the first two years and on every six months between 2nd and 5th year and annually after 5 years. Chimerism testing was performed more frequently in those with persistent MC. MC was defined as persistance of 5% to 95% residual recipient hematopoietic cells.

Full donor chimerism (FC) was defined as persistance of >95% donor hematopoietic cells (26).The intensity of conditioning regimens were classified according to the published criteria of the Center of International Blood and Marrow Transplant Research (CIBMTR) (5). T-cell depletion was not used. Overall survival (OS) after ASCT was defined as the duration from transplant date to the date of death or to the date of the latest follow-up for the survivors. Progression-free survival (PFS) after ASCT was described as the duration from the date of the transplant to the first date when there was a progression in disease or to the date of death or to the latest follow-up date for progression-free patients (27).

*PCR Amplification*

Unless stated otherwise, the protocols in the Identifiler Plus Kit User Guide. The PCR amplification reaction mixture was prepared in a volume of 25 µL containing 10 µL of AmpFℓSTR Identifiler Plus Master Mix, 5 µL of AmpFℓSTR Identifiler Plus Primer Set, and a maximum volume of 10 µL of target DNA. Samples were amplified in 96-well reaction plate in the GeneAmp PCR system 9700 with a gold-plated silver or silver block (Applied Biosystems). A two-step PCR cycling protocol was also optimized for the Identifiler Plus Kit. The standard thermal cycling conditions in the 9600 emulation mode consisted of enzyme activation at 95°C for 11 min, followed by 28 or 29 cycles of denaturation at 94°C for 20 sec and annealing⁄ extension at 59°C for 3 min. A final extension step was performed at 60°C for 10 min, followed by a final hold at 4°C if the PCR products were to remain in the thermal cycler for an extended time.

*DNA Separation and Detection*

PCR products were analyzed on an ABI 3130xl Genetic Analyzer using POP-4 polymer and 36 cm capillary. One microliter of amplified PCR products were added to 10.7 µL formamide and 0.3 µL GeneScan LIZ-500 for the Identifiler® Plus kit. The mixed solution was then denatured at 95 °C for 2 min. The injection condition was 15 kV/5 s. Alleles were called using GeneMapper® ID v3.2.1. Except for stutter analysis, an analytical threshold of 50 RFU and a stochastic threshold of 200 RFU, which has an allelic drop-out probability of 0.01, were used for all samples. For casework samples, consensus profiling was used to obtain a single profile and to account for allelic drop-ins. Alleles that were not repeatable were discarded. Mixtures were assessed using Caragine's et al. criteria (T. Caragine, R. Mikulasovich, J. Tamariz, E. Bajda, J. Sebestyen, H. Baum, M. Prinz, Validation of testing and interpretation protocols for low template DNA samples using AmpFlSTR® Identifiler® (28). Croat. Med. J. 50 (2009) 250–267) and removed from further analysis.

*Statistical Analysis*

The statistical analyses were performed with IBM SPSS Statistics v21 software. The significance level was 0.05. Groups were compared using nonparametric tests for continuous and the chi-squared statistic for categorical variables. The cases where Type-1 error level was under 5% was accepted as statistically significant.

## RESULTS

The 335 patients who were performed ASCT at our center and survived more than 3 months after transplantation were included. Median age was 34 years. There was 218 male and 117 female patients. 42 (12.5%) of these patients had MC. There were 32 male and 10 female mix chimeric patients. There were 319 patients with malignant hematological diseases and MC was seen 10.9% of these patients. The ratio of MC in benign hematological diseases was 43.75%. The distrubition of diseases in FC and MC groups is given in Table 1.

**Table 1**. The distrubition of diseases in FC and MC groups

| Disease | FC Group (n) (%) | MC Group(n)(%) | Total |
|---|---|---|---|
| **AML** | 130 (83.3%) | 26 (16.6%) | 156 |
| **ALL** | 93 (95.8%) | 4 (4.12%) | 97 |
| **NHL** | 12 (100%) | 0 | 12 |
| **HL** | 25 (92.5%) | 2 (7.4%) | 27 |
| **AA** | 8 (57.14%) | 6 (42.8%) | 14 |
| **CML** | 9 (90%) | 1 (10%) | 10 |
| **MM** | 7 (77.7%) | 2 (22.2%) | 9 |
| **CLL** | 4 (100%) | 0 | 4 |
| **PNH** | 1 (50%) | 1 (50%) | 2 |
| **MF** | 4 (100%) | 0 | 4 |
| **Total** | 293 | 42 | 335 |

AML: Acute Myeloid Leukemia, ALL: Acute Lymphoblastic Leukemia, NHL: Non Hodgkin Lymphoma, HL: Hodgkin Lymphoma, AA: Aplastic Anemia, CML: Chronic Myeloid Leukemia, MM: Multiple Myeloma, CLL: Chronic Lymphocytic Leukemia, PNH: Paroxysmal Nocturnal hemoglobinuria, MF: Myefofibrosis, FC: Full Chimerism, MC: Mix Chimerism

Median follow up of all patients was 33 months. During follow up period 127 (43%) of 293 patients in FC group and 11 (26.1%) of 42 patients in MC group died. 11 patients received DLI after MC detected, 4 of them converted into FC. 10 mix chimeric patients converted into FC spontaneously. In 3 patients no donor cell was observed in time (0%). 2 of them are still in remission. 25 patients remained mix chimeric during follow up period.

In our study, 66 patients received RIC regimen and 10 (15.1%) of them had MC during follow up period. 3 of these 10 mix chimeric patients who received RIC regimen had relapse disease and 2 of them died. 269 patients received MAC regimen and 32 (11.8%) of them had MC during follow up period. 14 of these 10 mix chimeric patients who received MAC regimen had relapse disease and 9 of them died. Lowest level of donor chimerism, time of first MC, time of lowest level of MC, relaps and mortality ratio, PFS and OS of mix chimeric patients were given in Table 2.

**Table 2.** Clinical outcome of patients who had mix chimerism during follow up

| Parameters | AML | AA | ALL | HL | MM | CML | PNH |
|---|---|---|---|---|---|---|---|
| **n** | 26 | 6 | 4 | 2 | 2 | 1 | 1 |
| **MC first detected month (median)** | 2 | 8 | 4 | 5 | 2 | 72 | 2 |
| **Lowest level of chimerism (%)** | 76 | 90 | 90 | 90 | 82 | 94 | 77 |
| **Lowest level detected month (median)** | 3 | 12 | 6 | 9 | 4 | 72 | 13 |
| **Relaps ratio** | 12/26 | 1/6 | 2/4 | 0 | 2/2 | 0 | 0 |
| **Mortality ratio** | 8/26 | 0 | 1/4 | 0 | 2/2 | 0 | 0 |
| **PFS (month)** | 12 | 27 | 28 | 27 | 5 | 75 | 36 |
| **OS (month)** | 37 | 44 | 34 | 27 | 13 | 75 | 36 |

AML: Acute Myeloid Leukemia, ALL: Acute Lymphoblastic Leukemia, NHL: Non Hodgkin Lymphoma, HL: Hodgkin Lymphoma, AA: Aplastic Anemia, CML: Chronic Myeloid Leukemia, MM: Multiple Myeloma, CLL: Chronic Lymphocytic Leukemia, PNH: Paroxysmal Nocturnal hemoglobinuria, MF: Myefofibrosis, MC: Mix Chimerism, PFS: Progression free survival, OS: Overall Survival

## DISCUSSION

The ability to detect relapse early in the post-transplant period is important to make treatment plan that can prevent disease recurrence and improve survival such as maintenance regimens or DLI. In addition determining chimerism may also be useful to monitor response to a DLI (29-31). There has been conflicting results regarding the correlation between disease relapse and chimerism levels after ASCT (31–34). In contrast to myeloablative transplants, RIC ASCT frequently results in varying degrees of MC (32, 33). Furthermore, MC may remain stable over time and may be together with prolonged remission, particularly in nonmalignant diseases, where MC may indicate a tolerant state associated with a low incidence of GvHD (36,37). Persisting MC was reported in 19 patients with hematological malignancies, with a median leukemia free survival of 12.5 years, and in patients with nonmalignant diseases, over a median period of 9.5 years after ASCT (38). In our study the mortality rate in MC patients was 26.1% and relapse ratio was 40.4%. 59.5% of patients with MC is still in remission. The mortality rate in MC patients with hematological malignancy was 31.4 % and relapse ratio was 45.7%. The relaps rate in MC patients with benign hematological diseases was 14.2%.and no patient with benign hematological disease died. In the study conducted by Reshef et al, they found that early donor-recipient chimerism levels predicted relapse in ASCT recipients who received a peripheral blood stem-cell graft after a uniform RIC regimen. They found that the risk for relapse at 1 year in patients who have chimerism levels of 90, 95 and 100% are 52, 34 and 21% respectively (21). In our study, 66 patients received RIC regimen and 10 of them had MC during follow up period. 3 of these 10 mix chimeric patients had relapse disease and 2 of them died. In the study conducted by Levrat et al, MC remained stable in 72% (23/32) of mix chimeric patients alive at 10 years (25). In our study, 11 patients received DLI after MC detected, 4 of them converted into FC. 10 mix chimeric patients converted into FC spontaneously. In 3 patients no donor cell was observed in time (0%). 2 of them are still in remission. 25 patients remained mix chimeric during follow up period. In our study we found that the mortality rate in full chimeric patients was higher than mix chimeric patients (43% vs 26.1%). This may be due to lower risk of GVHD in mix chimeric patients. In addition to this MC was observed more often in benign hematological diseases compared to malignant hematological diseases (43.75% vs 10.9%, respectively).

In conclusion; ASCT may result in MC especially after RIC regimens. MC does not always related with relapse risk and mix chimeric patients with malignant or benign hematological diseases can stay in remission for a long time. Our data suggests that MC is not necessarily linked to a worse prognosis as compared to patients with FC.

**Conflict of interest**

No conflict of interest was declared by the authors.

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