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Adult aplastic anemia patients can maintain remission after allogeneic hematopoietic stem cell transplantation in a mixed chimeric state

Erişkin aplastik anemi hastaları allojenik hematopoietik kök hücre nakli sonrası miks kimerik durumda remisyonda kalabilir

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Abstract

Aim: Allogeneic stem cell transplantation (allo-SCT) is performed as a curative treatment in young, fit, severe aplastic anemia (SAA) and very severe aplastic anemia (VSAA) patients. Age is one of the most significant factors that impact survival after transplantation. With advancing age, survival rates decrease, therefore it is crucial to perform allo-SCT without any delay in appropriate SAA and VSAA patients. Mixed chimerism (MC) in the post-allo-SCT period may be a sign of relapse or graft failure. In this study, we aimed to evaluate the outcome of allo-SCT in adult SAA and VSAA patients and the significance of chimerism monitoring in follow up.

Methods: The data of 16 adult AA patients who underwent allo-SCT at our center were included in this observational study. HLA match, conditioning regimens, chimerism and remission status were recorded.

Results: Median PFS was 70.4 months and median OS was 89.7 months in all patients. During the follow-up period, 9 patients remained fully chimeric whereas 6 patients had MC. The chimerism level of one patient, who had engraftment failure, was <5%. Both fully chimeric and mixed chimeric patients remained in remission.

Conclusion: More AA patients can be treated with allo-SCT with more optimized conditioning regimens that provide sustained engraftment with minimum toxicity. Our study showed that adult AA patients can maintain remission for a long time after allo-SCT, even in a mixed chimeric state.

Keywords: Adult aplastic anemia, Allogeneic stem cell transplantation, Chimerism

Öz

Amaç: Allojenik kök hücre nakli (AKHN), uygun vericisi bulunan fit ağır aplastik anemili (SAA) ve çok ağır aplastik anemili (VSAA)hastalarda küratif tedavi yöntemi olarak uygulanmaktadır. Yaş, nakil sonrası sağkalımı belirleyen en önemli faktörlerden biridir ve ilerleyen yaş ile birlikte sağkalım oranları azalmaktadır, bu nedenle uygun hastaların geciktirilmeden nakle alınması büyük önem taşımaktadır. AKHN sonrasında miks kimerizm (MK) olması nüs ya da engraftman yetersizliğinin işareti olabilir. Biz bu çalışmada, erişkin SAA ve VSAA hastalarında AKHN sonrasındaki durumunu ve takipte kimerizmin önemini incelemeyi amaçladık.

Yöntemler: Merkezimizde AKHN olan 16 AA hastası bu gözlemsel çalışmaya dahil edildi. HLA durumu, hazırlama rejimi, kimerizm ve remisyon durumu kaydedildi.

Bulgular: Tüm hastalarda medyan PFS 70,4 ay ve medyan OS 89,7 ay olarak izlendi. Takip süresi boyunca, 9 hasta tam kimerik iken 6 hasta MK ve 1 hastada kimerizm <%5 izlendi. Kimerizmi <%5 olan hastada engraftman kaybı izlenirken, diğer tam kimerik ve miks kimerik hastalar remisyonda izlendi.

Sonuç: Minimum toksisite ile engraftmanı sağlayan optimize hazırlama rejimleri kullanılarak daha fazla hasta allo-SCT ile tedavi edilebilir. Çalışmamız, yetişkin AA hastalarının allo-SCT'den sonra, miks kimerik bir durumda bile uzun süre remisyon sağlayabildiğini göstermiştir.

Anahtar kelimeler: Erişkin aplastik anemi, Allojenik kök hücre nakli, Kimerizm

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Introduction

Aplastic anemia (AA) is defined by hypocellular bone marrow and pancytopenia without bone marrow fibrosis and abnormal bone marrow infiltration. Its incidence in Europe is 2-3 / 1,000,000 annually. About 70-80% of cases are idiopathic. It has a biphasic age distribution and peaks between the ages of 10-25 [1].

According to the criteria of Camitta, at least two of the following values should be detected in complete blood count (CBC) for diagnosis of AA: Hemoglobin (Hb) <10g/dL, platelet $<50x10^9$/L, neutrophil $<1.5x10^9$/L [2]. Modified Camitta criteria is used to determine the severity of the disease. For the diagnosis of severe aplastic anemia (SAA), bone marrow cellularity must be less than 25% or if the cellularity is 25-50%, the ratio of residual hematopoietic cells must be less than 30%. In addition, at least two of the following values must be present in CBC: Absolute neutrophil count (ANC) $<0.5x10^9$/L, platelet count $<20x10^9$/L, reticulocyte count $<20x10^9$/L. The diagnosis is very severe aplastic anemia (VSAA) when the neutrophil count is $<0.2x10^9$/ L in peripheral blood in addition to the criteria of SAA [3].

Allogeneic stem cell transplantation (allo-SCT) is a curative treatment method in young, fit SAA and VSAA patients. Age is one of the most crucial factors which impact survival after transplantation, and with advancing age, survival rates decrease. Therefore, it is crucial to perform allo-SCT without any delay in SAA and VSAA patients [3].

Monitoring donor-patient chimerism is useful in evaluating engraftment status [4]. Especially after reduced-intensity conditioning (RIC) regimens and T-cell depletion, mixed chimerism (MC) may occur [5,6]. Increasing MC levels in the post-allo-SCT period may be a sign of disease relapse or graft failure. Conversely, decreasing MC may be a sign of graft versus host disease (GVHD) or graft-versus-tumor effect [7].

In this study, we aimed to evaluate the outcome of allo-SCT in adult age group SAA and VSAA patients and the importance of chimerism monitoring in the follow-up period.

Materials and methods

A total of 16 adult AA patients who underwent allo-SCT at our center between October 2009 and February 2020 were included. The data was analyzed retrospectively. Patients who were diagnosed with AA according to Camitta criteria and defined as SAA or VSAA according to the modified Camitta criteria were considered eligible for the study.

As conditioning regimen, 8 patients received CY-ATG [cyclophosphamide (50 mg/kg /day, 4 days) and anti-thymocyte globulin (ATG)], 2 patients received FLU-CY-ATG [Fludarabine (30 mg/m^2, 4 days), cyclophosphamide (20 mg/kg, 2 days), and ATG], 6 patients received FLU-CY-ATG-TBI [Fludarabine (30 mg/m^2, 4 days), cyclophosphamide (20 mg/kg, 2 days), ATG and total body irradiation (TBI) (2 gray on day -1)]. Bone marrow-derived stem cells were used in 11 patients and peripheral blood-derived stem cells were used in five. High-resolution typing of human leukocyte antigen (HLA) -A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQ was performed in all patients.

Post-transplant overall survival (OS) was identified as the time from transplantation until death or until the last follow-up in surviving patients. Post-transplant progression-free survival (PFS) was defined as the duration from transplantation until progression. Neutrophil engraftment was described as the first day that absolute neutrophil count (ANC) was >500/mm^3 without any granulocyte stimulating factor support for 3 consecutive days, and platelet engraftment was described as the first day that platelet count was >20000/mm^3 without any transfusion support for 3 consecutive days. Transplant-related mortality (TRM) was identified as deaths in the first 100 days after transplantation [8].

International Bone Marrow Transplant Registry (IBMTR) grading was performed to define the severity of acute graft versus host disease (GVHD) [9]. National Institute of Health (NIH) 2015 consensus criteria was used to define chronic GVHD severity [10].

Donor-patient chimerism was monitored from the peripheral blood on days 30, 60, 90, then, every 3 months in the first 2 years, every 6 months between the 2nd and 5th year and annually after 5 years. MC was defined as persistence of 5% to 95% remaining recipient hematopoietic cells. Full donor chimerism (FC) was defined as the persistence of >95% donor hematopoietic cells [11].

Statistical analysis

The data analyzed with SPSS software, V21.0 (SPSS Inc., Chicago, IL). Descriptive statistics were used to summarize the data. Categorical data were defined as ratios, and numerical data, as median (range: min-max). Kaplan-Meier test was used to estimate OS and PFS and log-rank test was applied to investigate factors affecting survival. $P<0.05$ was considered statistically significant.

Results

Characteristics of patients are presented in Table 1. Median PFS was 70.4 (95%CI: 44.89-95.91) months and median OS was 89.7 (95%CI: 67.96-111.39) months in all patients.

Table 1: Characteristics of patients

Characteristics	Patients
Gender (Male/Female)	8 male / 8 female
Age, median (range)	24 (18-53)
Source of stem cells	Bone marrow derived: 11
	Peripheral blood derived: 5
Donor type	Related: 12
	Unrelated: 4
HLA Match	Full Match: 12
	Mismatch: 3
	Haploidentical: 1

HLA: Human leukocyte antigen

In patients ≤24 years of age, between 25-39 years of age and ≥40 years of age, OS were 67.2 (95%CI: 46-88.3) months, 92.5 (95%CI: 59.4-125.6) months and 19.5 (95%CI:0-45.1) months, respectively. PFS were 51.4 (95%CI:26.6-76.1) months, 77.7 (95%CI: 40.2-115.2) months and 19.5 (95%CI:0-45.1) months, respectively. There was no statistically significant difference between age groups in terms of OS and PFS (P= 0.479 and P=0.729). The median duration between the date of diagnosis and allo-SCT was 7 months. Twelve patients had acquired AA, while 4 patients had Fanconi Anemia (FA). In acquired AA patients, median OS and PFS were 91.3(95%CI: 66.4-116.1) months and 75.3 (95%CI: 46.8-103.9) months, respectively. The same values were 30.3(95%CI: 13.7-46.8) months and 30.3 (95%CI: 6.8-53.7) months, respectively, in FA

patients. The median EBMT score was 2 (range: 0-5) and the median Sorror comorbidity score was 0 (range: 0-3). The median number of CD34+ stem cells was 3.17×10^6/kg (range: 1.62-7.26×10^6/kg) in patients who received bone marrow-derived stem cells and 5.26×10^6/kg (range: 4.23-6.40×10^6/kg) in patients who received peripheral blood-derived stem cells. The median platelet engraftment was 34 (range: 31-39) days in FA patients and 17 (range: 14-34) days in those with acquired AA. The median neutrophil engraftment was 14 (range: 12-15) days in FA patients and 17 (range: 14-28) days in patients with acquired AA.

During the follow-up period, 9 patients remained fully chimeric while 6 patients had mixed chimerism and 1 patient had a chimerism level of <5%. The chimerism level of one patient, who had engraftment failure, was <5%. Both fully chimeric and mixed chimeric patients remained in remission.

TRM was 12.5%. Median platelet engraftment was 34 (range: 31-39) days in FA patients and 17 (range: 14-34) days in acquired AA. Median neutrophil engraftment was 14 (range: 12-15) days in FA patients and 17 (range: 14-28) days in acquired AA.

Severe acute hepatic GVHD was observed in 1 patient who received peripheral blood-derived stem cells. Severe chronic GVHD was not observed in anyone.

Discussion

Allo-SCT is the curative treatment option for fit SAA and VSAA patients. Performing allo-SCT in adult age group AA patients is particularly important as survival rates decrease with advancing age [3]. Although allo-SCT is the standard approach in fit SAA and VSAA patients, there is no standard conditioning regimen. The optimal conditioning regimen was investigated by many researchers to provide continuous engraftment with minimal complications. In a previous study, in allo-SCTs performed in matched siblings using CY conditioning regimen, graft failure was observed at a high rate, especially in severely transfused patients [12]. Lower incidence of graft failure was found when CY-TBI was used as a conditioning regimen in allo-SCT from fully matched sibling donors, but in the long-term follow-up, higher morbidity and mortality incidences were observed [13-16]. Many researchers performed allo-SCT from sibling donors using CY- ATG conditioning regimen [13,17-19]. In a phase II prospective study by the Seattle group, in AA patients who received CY- ATG conditioning regimen, 3-year OS was 92%. This OS was significantly higher than the historical control group that received CY conditioning regimen alone (92% vs 72% in 3 years, P=0.043) [17]. In a large study based on the data of the Center for International Blood and Marrow Transplant Research (CIBMTR) comparing the results of patients using peripheral blood-derived stem cells and bone marrow-derived stem cells, a higher rate of chronic GVHD (27% vs 12%, P=0.002) and lower OS duration (5 year OS 73% vs 85%, P=0.024) was determined in patients who received peripheral blood-derived stem cell [20]. In our study, grade 3-4 chronic GVHD was observed in 2 patients. One of these patients received bone marrow-derived stem cells while the other received peripheral blood-derived stem cells. Severe acute GVHD was observed in none.

In the analysis by CIBMTR, the incidence of neutrophil engraftment was similar among all age groups, whereas the incidence of platelet engraftment was significantly lower in patients older than 40 years of age (P=0.010) when compared to patients ≤20 years of age, but no statistically significant difference was found compared to those aged 20 to 40 years (P=0.098) [21]. In our study, median platelet engraftment was 34 (range: 31-39) days in FA patients and 17 (range: 14-34) days in acquired AA. Median neutrophil engraftment was 14 (range: 12-15) days in FA patients and 17 (range: 14-28) days in acquired AA.

In the analysis by CIBMTR, the incidence of mortality was significantly higher in patients older than 40 years of age compared to those aged under 20 years (P<0.001) and those aged between 20 to 40 years (P=0.008) [21]. In our study, TRM was 12.5%. In the phase II prospective study of The European Society for Blood and Marrow Transplantation (EBMT) analyzing the results of patients who underwent allo-SCT from sibling donors using FLU-CY-ATG conditioning regimen, no significant difference was detected in terms of OS between patients aged ≥40 years and 30-40 years of age [22]. In the study evaluating the results of 82 SAA patients who underwent allo-SCT from sibling donors using the FLU-CY-ATG conditioning regimen, no significant difference was found in terms of OS in patients aged <20 years, 20-39 years, 40-49 years, and 50-59 years of age (88%, 97%, 92% and 86%) [23]. In our report, we found no differences regarding OS and PFS when patients were classified according to their ages as ≤24 years, 25-39 years and ≥40 years.

In a phase II prospective study by EBMT, the incidence of graft failure was 18%, and 2-year OS was 73% in patients who underwent allo-SCT from unrelated donors using FLU-CY-ATG conditioning regimen. In this study, in patients older than 14 years, the incidence of graft failure was higher (32% and 5%, P=0.030), and OS was lower (61% and 84% in 2 years, P=0.2) [24]. In addition, examination of the results of patients who underwent allo-SCT from unrelated donors using FLU-CY-ATG- ± low dose TBI from the EBMT data yielded no significant difference in OS durations between patients older and younger than 27 years of age (5 year OS; 78 vs 79%, p> 0.050) [24].

There have been conflicting results regarding the correlation between disease relapse and chimerism levels after allo-SCT [25-28]. RIC allo-SCT often results in varying degrees of MC, compared to myeloablative transplants [25,26]. In addition, MC can maintain steadiness and might be associated with sustained remission, especially in nonmalignant diseases, where MC may imply a tolerant condition associated with low frequency of GVHD [29,30]. In our study, through the follow-up period, 9 patients remained fully chimeric whereas 6 patients had mixed chimerism. The chimerism level of one patient, who had engraftment failure, was <5%. Both fully chimeric and mixed chimeric patients remained in remission.

Limitations

The limitations of the study were small and heterogenous cohort with varying donor types and dissimilar sources of stem cells received from allo-SCT.

Conclusion

Allo-SCT is a curative treatment method in patients with suitable donors and no serious comorbid conditions in SAA and VSAA patients, but there is no standard conditioning regimen. More AA patients can be treated with allo-SCT using more optimized conditioning regimens that provide sustained engraftment with minimum toxicity. In our study, mix chimeric adult AA patients remained in remission after allo-SCT during the follow-up period. Mechanisms of AA remission with mixed chimerism warrants further investigation.

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