EFFECTS OF CHIMERISM ON GRAFT-VERSUS-HOST DISEASE, DISEASE RECURRENCE, AND SURVIVAL AFTER HLA-IDENTICAL MARROW TRANSPLANTATION IN IRAN

Kamran Alimoghaddam MD, Hamid Ghaffari PhD, Forough Foroughi MD\*, Bahram Chardouli MSc, Zohreh Sanaat MD, Babak Bahar MD, Asadollah Mousavi MD, Masoud Iravani MD, Ardeshir Ghavamzadeh MD

Background: The coexistence of recipient's and donor's hematopoietic systems after allogeneic marrow transplantation is called mixed chimerism.

Objective: The objective of this study was to evaluate the effects of MC on graft-versus-host disease (GVHD), disease recurrence, and survival after HLA identical marrow transplantation in a transplant center in Iran.

Methods: The association of MC with acute GVHD, disease recurrence, survival, and relapse-free survival was investigated in 91 patients who underwent either bone (n = 12) or peripheral blood (n = 79) HLA-identical marrow transplantation. Chimerism was assessed using multiplex amplification of short tandem repeats (STR). Patients had thalassemia (n = 19), acute myelogenous leukemia (AML) (n = 29), acute lymphocytic leukemia (ALL) (n = 20), chronic myelogenous leukemia (CML) (n = 18), and other diseases (n = 5). The median age was 21 (range: 3 – 50) years. There were 38 (42%) female and 53 (58%) male participants. Conditioning was made through busulfan plus cyclophosphamide in 34 patients; busulfan plus fludarabin in 51 patients; and busulfan plus fludarabin plus antithymocyte globulin in 6 patients. The median follow-up was 13 months.

Results: On day +30, complete chimerism (CC) was observed in 72 (79%) patients, MC in 15 (17%), and no chimerism in 4 patients. The incidence of acute GVHD was significantly (P = 0.01) lower in mixed chimeras than in complete chimeras. There was no significant difference in acute GVHD grade (I, II vs. III, IV) between the two groups. The incidence of relapse was 18%. There was no difference in relapse rate between MC and CC groups. Overall survival was 89%. There was no significant difference in the overall survival between MC and CC group (96% vs. 85%, respectively). Relapse-free survival was 80% that was not significantly different between the two groups.

Conclusion: Despite some previous reports, we found no significant difference in the survival and relapse rates between MC and CC groups.

Keywords: Allogeneic bone marrow transplantation • chimerism • STR-PCR

Introduction

During the last three decades, allogeneic bone marrow transplantation (BMT) has been extensively used to treat patients with certain hematological diseases. The major causes of treatment failure are disease relapse, graft rejection, and graft-versus-host disease (GVHD).

One of the main goals of posttransplantation monitoring is to predict these unwanted events so that we could set up the appropriate preventive measures. In this context, mixed chimerism (MC) quantification has been proposed as an important method in monitoring post-BMT outcome.\(^1\) The term “chimerism” refers to the presence of lym-
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phohematopoietic cells of nonhost origin. Full or CC generally refers to complete replacement of host by donor lymphohematopoiesis. MC indicates the presence of both donor and recipient cells within a given cellular compartment, e.g., lymphocytes. The characterization of an increase in the proportion of host cells in the post-BMT period strongly suggests a risk of disease recurrence. In such condition, early diagnosis is of paramount importance on the patient outcome, since relapsing patients may enter durable second remission through a donor lymphocyte infusion. Chimerism analysis provides a rational method of assessing the ability of different conditioning regimens, GVHD, prophylactic regimens, and cellular therapy, to promote engraftment and graft-versus-leukemia activity.

Very early hematopoietic stem cell transplantation (HSCT) studies recognized the importance of establishing chimerism. Early investigators, however, had to rely on techniques such as red blood cell phenotyping, immunoglobulin isotype analysis, and cytogenetics to assess the chimeric state. Limitations of these techniques include limited degrees of polymorphism, poor sensitivity, and the requirement for a donor and recipient who are sex mismatched.

The most generally applicable and useful methods to evaluate chimerism are DNA techniques such as polymerase chain reaction (PCR)-based assays of polymorphic mini- or micro-satellite markers, permitting sensitive assessment of host hematopoiesis after marrow transplantation, which allows the detection of minor populations of donor or recipient cells. Information on this persistence and the cell lines in which it occurs may permit therapeutic intervention in patients at high risk for rejection and/or relapse. The objective of this study, therefore, was to evaluate the effects of MC on GVHD, disease recurrence, and survival after HLA-identical marrow transplantation in a transplant center in Iran.

Materials and Methods

The association of MC with acute GVHD, disease recurrence, survival, and relapse-free survival was investigated in 91 patients who underwent either bone (n = 12) or peripheral blood (n = 79) HLA-identical marrow transplantation at the Hematology-Oncology and BMT Research Center, Shariati Hospital, Tehran, Iran. The patients’ characteristics are listed in Table 1.

DNA preparation

DNA analysis was performed on the peripheral blood leukocytes of donors and patients before and after BMT. Mononuclear cells and polymorphonuclear cells (granulocytes) were isolated by Ficoll-Isopaque density gradient centrifugation of cells and DNA was isolated by the salting out method. DNA was also extracted, using silica membranes (QiAmp blood Kit; Qiagen, Hilden, Germany), as recommended by the manufacturer. DNA concentration was measured by a UV spectrophotometer at wavelength of 260 nm.

Chimerism analysis

The method used to assess chimerism was polymerase chain reaction of short tandem repeats (PCR-STR). Three autosomal tetra-nucleotide STR loci with nonoverlapping allele size range were simultaneously amplified. Loci were ARA, ADA, D4S2366, D16S539, D7S820, D13S317, F13A1, FES/FPS, VWA, CSF1PO, TPOX, and TH01. All the markers used were amplified under identical PCR conditions. Six percent polyacrylamide gels were used and DNA was visualized using a DNA silver staining system. Calculation of the degree of MC was based on the percentage of patient and donor loci in the patients.

CC, CM, and “no chimerism” (NC) were considered when there were >95%, 5 – 95%, and <5% of donor cells were found in the recipient, respectively.

Table 1. Characteristics of participants.

<table>
<thead>
<tr>
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<td>CsA + MTX</td>
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</table>

ALL = acute lymphocytic leukemia; AML = acute myelogenous leukemia; CML = chronic myelogenous leukemia; Thal = thalassemia; FA = fanconi anemia; Bu = busulfan; CTX = cyclophosphamide; Flu = fludarabine; ATG = antithymocyte globulin; CsA = cyclosporine A; MTX = methotrexate.
Statistical analysis
Statistical analyses were conducted using the SPSS statistical software. The data on survival and relapse-free survival were determined, using the Kaplan-Meier survival analysis with 95% confidence intervals and drawn out curves. Relationship of chimerism and other factors like patient’s and donor’s age and sex, the conditioning regimen, the GVHD prophylaxis, marrow cell type, and the number of infused cell was determined using Student’s $t$-test and $\chi^2$ test.

Results
The median follow-up for patients was 13 months. On day 30 post-BMT, CC was observed in 72 (79%) patients, MC in 15 (17%), and NC in 4 (5%) patients. STR analysis was made for 30 patients on day +60. Status of two patients changed from CC to MC; three from MC to CC; five patients remained MC; and 20 remained CC groups. The incidence of acute GVHD in our patients was 58%. This was significantly ($P = 0.01$) lower in MC than in CC. There was no significant difference in acute GVHD grade (I, II vs. III, IV) between the two groups; thus, the severity of acute GVHD was not related to the degree of chimerism. Sixteen (18%) patients experienced relapse; there was no significant difference in the relapse rate between patients with MC and CC. Nine patients died including one with MC and 8 with CC. The overall survival rate was 89% (Figure 1). There was no statistically significant difference in the overall survival between patients with MC and CC (96% vs. 85%, respectively) (Figure 2). Relapse-free survival was 80% that was not significantly different between the two groups.

There was no relationship between the degree of chimerism and patient’s and donor’s age and sex, the conditioning regimen, the GVHD prophylaxis, marrow cell type, and the number of infused cells.

Discussion
The importance of analyzing chimerism after allogeneic marrow transplantation is to help predicting transplantation outcome and to implement early therapeutic interventions. Approaches to the measurement of lymphohematopoietic chimerism have evolved from bench research to clinical tools. In this study, we used peripheral blood sample to analyze chimerism, because based on previous studies, peripheral blood cells are generally more useful than bone marrow cells for chimerism analysis and it is easier for the patients.

In our study, we used multiplex amplification of STRs as the most accurate and sensitive method to analyze chimerism. The percentage of MC and CC in our patients were similar to other studies, but several groups have suggested that MC is associated with an increased risk of disease relapse. In our study, we found no significant
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Figure 2. The overall survival in patients with CC, MC, and NC.

Figure 2. The overall survival in patients with CC, MC, and NC.

We therefore, recommend that: 1) chimerism analysis should be undertaken using sensitive and informative techniques. Currently, STR-PCR analysis is the most appropriate approach; 2) more frequent analysis should be carried out. A working schedule of 1, 3, 6, and 12 months seems to be reasonable; 3) the early patterns of chimerism could be predictive of either GVHD (increasing donor chimerism) or graft loss (declining donor T-cell chimerism to ≤20% donor cells). In the case of graft loss, the use of donor lymphocyte infusion should be considered.

References


9 Elmaagachi AH, Beelen DW, Becks HW, et al. Molecular studies of chimerism and minimal residual disease after allogeneic peripheral blood progenitor cell or bone


