Non-Myeloablative Stem Cell Transplantation in Hematologic Malignancies: An Experience from the Hematology-Oncology and BMT Research Center

Keyhanian S.[1], Ghavamzadeh A.[2], Bahar B.[2], Alimoghaddam K.[2], Shamshiri AR.[3], Gholibeikian.S.[4]

[1]*Oncologist-Hematologist, Azad Eslami University of Medicine, Tonekabon,*
[2]*Oncologist-Hematologist, Hematology- Oncology and BMT Research Center Tehran University of Medical Sciences,*
[3]*Student of epidemiology, Hematology- Oncology and BMT Research Center Tehran University of Medical Sciences,*
[4]*Data manager, Hematology- Oncology and BMT Research Center Tehran University of Medical Sciences*

Abstract:
Background: Myeloablative-allogeneic stem cell transplantation is a common way of treating various malignant and nonmalignant diseases; but, it is associated with hazardous immediate and late complications. The majority of patients are not good candidates for high dose therapy because of old age, medical co-morbidities or previous heavy treatments. The donor stem cells can engraft in the recipient and induce mixed chimerism when we use a less intensive, but sufficiently immunosuppressive, conditioning regimen, known as mini-transplantation or non-Myeloablative allogeneic Stem Cell Transplantation (NM-allo-SCT).
Methods: The conditioning regimens were the combination of Fludarabine and Cyclophosphamide or Busulfan and ATG. Prophylaxis against graft versus host disease (GVHD) included Cyclosporine A (CSA) +/- Methotrexate. A multiplex-PCR using short tandem repeats (VNTR) was used for chimerism analysis.
Results: We report the results of NM-allo-SCT from the HLA-identical siblings in 20 patients with AML (N=7), CML (N=6), NHL (N=2), MDS (N=2), ALL (N=1) and Fanconi anemia (N=2). Fourteen males and 6 females with median age of 43 years (range 8-55) underwent NM-allo-SCT and were followed up 4-870 days (median 420 days). Typical side effect of conventional HSCT, such as severe mucositis, vomiting and VOD were absent. Most of the patients did not become severely pancytopenic and had relatively short hospitalization. Hematological recovery was rapid, a median of 8.5 days. Acute GVHD (grade ≥II) and extensive chronic GVHD was observed in three patients. Most of the patients initially had mixed-chimerism, progressing to full-donor-chimerism in 11 patients, after the interruption of the CSA therapy, and, in one patient, after DLI. Nine patients died, six from relapse or disease progression and three from transplantation-related complications (GVHD, infection or secondary malignancy). 14 month overall survival and disease free survival of 55% and 50%, respectively, was observed.
Conclusion: Our results confirm that NM-allo-SCT is safe and minimally toxic and is a potential new approach for a safer treatment of a large variety of hematologic diseases, especially in patients with AML and CML in remission.

Keywords: Hematopoietic stem cell transplantation, Allogeneic, Non-myeloablative, Adverse effects, Graft vs Host Disease, Survival rate

Introduction

Conventional allogeneic hematopoietic stem cell transplantation (allo-HSCT) with myeloablative conditioning is a common modality in treating various hematologic malignancies and genetic diseases resistant to a conventional dose of chemotherapy.

Myeloablative allo/HSCT is associated with hazardous immediate and late complications which generally restrict such a potentially curative treatment in patients <50-55 years of age with normal organ function.[1]

In patients with an underlying hematologic disorder, the transplantation procedure was considered mostly as a rescue procedure after myeloablative conditioning to eradicate the basic disease.

But, more than two decades ago, it was related that, the Graft Versus Tumor (GVT) or Graft Versus Leukemia (GVL) effect can be induced by T Lymphocyte- enriched donor derived stem cell.[2, 3]

The powerful GVL effect was strengthened with infusion of variable donor lymphocytes (DLI).

In recent years, donor allogeneic stem cell engraftment in recipients has been achieved by useing less intensive, but sufficiently immunosuppressive, conditioning regimens that induce mixed chimerism.

The majority of patients with hematologic diseases are not good candidates for myeloablative HSCT due to old age, medical co-morbidities, poor organ function or previous heavy treatments.

These patients can be treated with a nonmyeloablative conditioning regimen also known as a mini-transplantation or Non-Myeloablative

allogeneic Stem Cell Transplantation (NM-allo-SCT).[4]

The goal of this study is to determine whether this regimen has low toxicity, in addition to its GVL effects, which are important in inducing the formation of alloreactivity against the host leukemic cells.

We have performed a prospective study to evaluate mixed chimerism, the incidence of common transplantation related complications (including acute and chronic Graft vs. Host Disease (GVHD), mucositis, veno occlusive disease (VOD), pulmonary complication, CMV infection) and the duration of overall and disease free survival.

Patients and Methods

We report the results of a nonmyeloablative conditioning regimen followed by an allogeneic peripheral blood stem cell (PBSC) transplantation from HLA-identical sibling donors in 20 patients with a hematologic disease who were included in a prospective study from 2001 to 2003 at the Hematology-Oncology and Bone Marrow Transplantation Research Center.

Eighteen patients had hematologic malignancies including 7 with acute myelogenous leukemia (6 in the first complete remission and 1 with persistent disease), 6 with chronic myelogenous leukemia in the first chronic phase, 2 with myelodysplastic syndrome (1 with refractory anemia, 1 with excess blast), 2 with non-Hodgkin's lymphoma (1 with Burkitt's lymphoma in second, complete remission, 1 with a mixed and large cell lymphoma resistant to chemotherapy), 1 with acute lymphoblastic leukemia (ALL) resistant to chemotherapy.

Two patients with Fanconi's anemia were also included (Table 1). Patients' ages ranged between 8 and 55 (median, 43) years.

Some patients were considered poor candidates for conventional allo-SCT because of old age (N=8), concurrent medical condition (N=5) and heavy treatment (N=7).

Prophylaxis against pneumocistis carini included trimethoprim/ sulfomethoxazole. Prophyloxis against the herpes simplex virus included low dose oral acyclovir.

Prophylaxis against GVHD included cyclosporine A (CSA) alone or CSA plus short course prevention by methotrexate (MTX). CSA was started three days before transplantation,

Table 1- Patients characteristic

No.	Age	Diagnosis	sex	HLA type
1	20	AML/1st CR	M	HLA-Matched
2	46	AML/1st CR	M	siblings
3	43	AML/1st CR	M	
4	26	AML/1st CR	M	
5	47	AML/2nd CR	M	
6	43	AML/2nd CR	M	
7	36	AML/Persistent disease	M	
8	48	CML/CP	F	HLA-Matched
9	55	CML/CP	F	siblings
10	47	CML/CP	F	
11	50	CML/CP	M	
12	42	CML/CP	M	
13	43	CML/CP	M	
14	46	MDS/RA	F	HLA-Matched
15	54	MDS/RAEB	M	siblings
16	24	All/2nd Relapse	F	HLA-Matched siblings
17	14	Burkitt's lymphoma/2ndcR	M	HLA-Matched
18	27	Mixed small and large cell Lymphoma / Persistent	M	siblings
19	8	Fanconi anemia/AA	F	HLA-Matched
20	18	Fanconi anemia/AA	M	siblings

initially as an intravenous infusion with a daily dose of 3mg per kilogram daily, switching to an oral dose of 6.25mg/kg 2 times a day, tapered as rapidly as possible after day 30.

The conditioning regimens for myeloid disease consisted of fludarabine 30 mg/m^2 intravenously (IV) on days –9 to –5 with busulfan 4 mg/kg IV on days –6 to –5 and anti T lymphocyte globulin (10mg/kg) for 4 consecutive days (day-4 to-1).

The conditioning regimen for lymphoid disease consisted of cytoxan 60mg/kg on days -7 to -6 followed by fludarabine 30mg/m^2 for 5 consecutive days (-5 to -1).

Two patients with Fanconi's anemia received cytoxan 20mg/kg with fludarabine and ATG.

G-CSF-mobilized peripheral blood stem cells (PBSC) were collected by leukapheresis after administration of G-CSF for 5 days then PBSC were counted for CD34 and CD3.

The total number of nucleated cells infused on day 0 ranged between 1.059 and 9.03 (mean 4.44)$\times 10^8$ /Kg.

After transplantation, serial samples of blood and marrow were analyzed to study chimerism using a variable number of tandem repeats (VNTR) on days 28, 56, 84 and 120 post transplantation.

Cytomegalovirus (CMV) antigenemia was monitored weekly until day +100 and its reactivation was treated with ganciclovir.

Results

The twenty studied patients consisted of 14 males and 6 females.

The median age at the time of transplantation was 43 years (range 8-55).

The median follow-up was 420 days (range 4-870).

All patients received a non-myeloablative conditioning regimen with fludarabine plus CTX (N=11) or busulfan and ATG (N=9).

Graft-Versus-Host disease (GVHD) prophylaxis consisted of a short course of prevention by MTX and CSA in 8 patients and CSA alone in 11 patients.

Median dose of mononuclear cell was 4.44×10^8/Kg.

Early transplantation-related toxicities were mild in most cases. 15 patients maintained oral intake throughout the transplantation.

Sever vomiting and veno-occlusive disease (VOD) of the liver were not observed in these patients.

Grade 2 mucositis and hemorrhagic cystitis were observed in only 1 patient.

Febrile neutropenia was documented in 13 patients but only 5 cases of positive blood culture and in 3 cases positive urine culture were observed.

Nine patients had reactivated CMV, 7 were asymptomatic and 2 developed pneumonitis. They all responsded to ganciclovir.

Pulmonary toxicity was observed in 5 patients.

Acute GVHD grades I to IV occurred in 13 patients, (grade I in 10 patients, grade II in 2 patients and grade IV in one patient).

Severe GVHD (grade IV) was observed in one patient with MDS (RA). This was the only cause of mortality in this patient.

Acute GVHD developed in 2 patients following sudden discontinuation of CSA and in 11 patients during CSA maintenance therapy.

Chronic GVHD was observed in 9 patients; 6 patients developed limited chronic GVHD and 3 patients had extensive chronic GVHD.

New malignancy (lymphoblastic lymphoma) occurred in one patient with Fanconi's anemia who had achieved 100% donor chimerism. the patient died on day +90.

Myositis developed in one patient with CML on day +150.

The common transplantation related complications of these 20 patients who underwent non-myeloablative allogeneic SCT are listed in Table 2.

Absolute neutrophil count (ANC) decreased to $<0.5\times10^9$/L in 16 patients, and recovered at a median of 8.5 (range 0-12) days after transplantation in 14 patients.

Two patients with refractory leukemia at the time of transplantation died on days +4 and +10 and were considered unevaluable for engraftment.

Platelet counts did not decrease to $<20\times10^9$/L in 6 patients and in the remaining 14 patients was recovered to $>20\times10^9$/L at a median of 4 (Range 0-14) days post transplantation.

14 patients required 0-14 (median 2.5) units of red blood cell transfusion.

Median, single, donor platelet transfusion was 2 units (range 2-10).

Donor chimerism was complete at least on one occasion in 12 (60%) patients and mixed in 4 (20%) patients.

In 11 patients, mixed chimerism progressed to full donor chimerism after discontinuation of CSA therapy. One patient received donor lymphocyte infusion to establish complete donor chimerism.

After 4-870 days of follow-up, 11 patients (AML=6, CML=5) remained alive. 10 of them are in complete remission with a full chimerism and karnofsky performance score of 80-100.

One patient with CML in chronic phase is alive at +810 post-transplantation.

Nine patients died, six from relapse or disease progression and three from transplantation-related mortality.

Disease progression occurred in MDS (RAEB), ALL, AML and NHL with persistent disease (N=1 each). These patients died on days +135, +4, +10, +70 post-transplantation respectively.

Relapse was observed in two patients with CML and Burkitt lymphoma. These patients died on days +690 and +80 post-transplantation respectively.

The causes of transplantation-related mortality included GVHD, secondary malignancy and pulmonary infection (N=1 each).

Two patients with Fanconi's anemia died of transplantation-related complications at days +90 and +160 post-transplantation.

With a median follow-up of 420 days, overall survival and disease free survival were 55% and 50% respectively.

Discussion

Our retrospective study shows that a non-myeloablative regimen is safe and minimally toxic. Typical side effects of conventional HSCT, such as severe mucositis, vomiting and VOD, were not seen. Most of the patients did not become severely pancytopenic and had relatively short hospitalization. The transfusion requirements were decreased. The risks of early bacterial infection and CMV reactivation were decreased.

Severity of acute GVHD in most of the patients was moderate. The initial chimerism was mixed, progressing to full-donor chimerism in 11 patients after discontinuation of CSA therapy and in one patient with DLI.

Post-transplantation course of patients with AML in first complete remission and CML in chronic phase at the time of transplantation was good. Sustained remission with full-donor chimerism were achieved in 10 of 13 patients with myeloid leukemia.

The previous studies demonstrate some major differences among malignancies, in their susceptibility to graft versus leukemia (GVL) effects and their sensitivity to non-myeloablative allogeneic transplantations.

CML has been the disease in which GVL effects are well documented. AML in complete remission is an indolent disorder that is not immediately life threatening, thus giving time for a GVL effect to develop.

The outcome of patients with refractory disease at transplantation (N=4) and Burkitt lymphoma on second complete remission (N=1) was poor. These patients died from disease progression.

High grade lymphoma and chemo-refractory diseases appear relatively insensitive to GVL effects and the rapid rate of proliferation of these malignancies may also outpace a developing GVL effects.

Two patients with Fanconi's anemia (FA) died from transplantation-related causes. FA patients are also considered to be at relatively high risk for mortality with lower doses of cyclophosphamide and ATG.

We should emphasize that our study was small and the follow-up was relatively short. Additional patients and more time will be required to determine the response to NM-SCT.

An important limitation is the prolonged time requirement for the induction of GVT effects.

Patients with rapidly progressive diseases, who are unlikely to live long enough for the induction of GVL effects, would not benefit from such therapy. Because of these limitations, NM-SCT should remain as an investigational approach for treatment of rapidly progressive diseases, but this modality is a useful therapeutic option for patients with acute myeloid leukemia in CR and CML in CP at the time of transplantation.

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