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Allogeneic Haemopoietic Stem Cell Transplantation Using Non-Myeloablative Conditioning - A Local Experience

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Summary

Allogeneic haemopoietic stem cell transplantation was initially considered as a means of delivering supralethal doses of chemotherapy with or without total body irradiation for the treatment of malignancy. However, it has become clear that this mode of therapy does not eradicate the malignancy in many patients and its benefit is largely due to the immune mediated graft versus malignancy effect. This has led to development of alternative strategy to utilize a less intensive preparative regimen pre-transplantation that provides sufficient immunosuppression to achieve engraftment of an allogeneic stem cell graft, thus allowing the evolution of a graft versus malignancy effect post-transplantation.

Since September 1999, we had carried out 10 cases of allogeneic peripheral blood stem cell transplantation: one case of aplastic anaemia, four cases of acute myeloid leukemia (AML) in first remission, and five cases of chronic myeloid leukemia (CML) in chronic phase. The preparative regimen was non-myeloablative comprising Fludarabine with Cyclophosphamide or Busulphan. Recovery from transplantation was rapid with no or brief period of neutropenia or thrombocytopenia. Engraftment was established by determining donor's short tandem repeats in the recipient's bone marrow at day 30,60 and 100 post-transplantation. Seven cases (70%) show partial or complete donor's chimerism by day 30 indicating successful engraftment. No treatment mortality was noted at day 100. Graft versus host disease was generally limited. Up to the date of reporting, two patients with CML had graft failure, one was successfully re-transplanted later. Two patients with AML had since relapsed and passed away. The others remain alive and well. The cost of transplantation on average was estimated to be about a quarter of that using a myeloablative regimen. It appears that this treatment strategy is a promising approach for the management of blood disorders.

Key Words: Non-Myeloablative Conditioning, Peripheral blood stem cell

Introduction

Allogeneic haemopoietic stem cell transplantation is an effective treatment for haematologic malignancies. The curative power of allogeneic haemopoietic stem cell transplantation was initially thought to be due to the cytoreductive

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effect of supralethal doses of the conditioning regimen consisting chemotherapy with or without radiotherapy. Retrospective analysis has demonstrated that the major benefit of the conditioning regimen for allogeneic haemopoietic stem cell transplantation is due to the immunosuppression of the recipient to permit donor haematopoietic engraftment and thereby establishing a graft versus malignant effect. Moreover, in patients with relapsed leukemia after allogeneic haemopoietic stem cell transplantation, infusion of donor lymphocytes restore remission. This observation suggests that the curative potential of allogeneic haemopoietic stem cell transplantation is due to the adoptive immunotherapy of the donor graft and not the conditioning regimen. Thus the concept of using non-myeloablative transplantation (NMT) to capitalize on donor mediated immunotherapy was introduced and with this, hopefully will reduce the treatment related mortality associated with intensive myeloablative regimens.

There were several non-myeloablative conditioning regimens. Most of them employed a combination of potent immunosuppressive purine analogs (e.g Fludarabine) along with either low dose total body irradiation (TBI) or intermediate-dose chemotherapy with or without anti-thymocyte globulin.

Materials and Methods

Since September 1999 until October 2001, 10 patients had consented to undergo NMT using peripheral blood stem cell (4 patients with AML in 1st complete remission (CR), 5 patients with CML in chronic phase and 1 patient with severe aplastic anaemia).

Conditioning regimens
Conditioning therapy before infusion of peripheral blood stem cell include immunosuppressive treatment with 3 daily infusion of Fludarabine 30mg/m^2/day and Cyclophosphamide 300mg/m^2 (day -3 to day -1) for the first 2 patients. 6 patients received infusion of Cyclophosphamide 60 mg/kg for 2 consecutive days (day -7 to day -6) follow by Fludarabine 25mg/m^2 for another 5 days (day -5 to day -1). In one patient with CML who had failed the 1st transplant, she was given Fludarabine 30mg/m^2 for 5 days (day-7 to day -3) and oral Busulphan 2mg/kg/day for 2 days (day -2 to day -1) at 2nd transplant, and so as the remaining 2 patients.

Supportive care
All patients were treated in an isolation room. Patients received prophylaxis with Ciprofloxacin 250 mg twice daily, Fluconazole 100mg twice daily, Bactrim 2 tablets twice daily and Acyclovir 200mg 3X / day. Broad spectrum antibiotics were started for temperature more than 38°C for two occasions or clinical signs of infection. These patients also received GM-CSF 5µg/kg/d from day+1 until engraftment.

Prophylaxis and treatment against GVHD
Prophylaxis against GVHD consisted of oral Cyclosporin 3mg/kg twice daily. Corticosteroid therapy (Prednisolone) was added 2mg/kg/d in patients with acute GVHD. Tapering of the dose of Cyclosporin was initiated after transplantation in patients who did not have signs or symptoms of chronic GVHD.

Stem Cell Collection and Transplantation
Donor received Filgrastim 300µg subcutaneously twice daily starting 4 days before 1st collection, stem cell harvesting was performed daily starting on day 5 of Filgrastim injection using Cobe Spectra cell separator until greater than 2x10^6 CD34+ cells / kg recipient weight were collected. Donor cells were kept at 4°C before infusion on the next day or cryopreserved with the standard technique if the transplantation is scheduled at a later date. Cryopreserved units were stored in liquid nitrogen and maintained in vapor phase until the time of transplantation. The cryopreserved units were thawed on the day of transplant. After thawing, bacterial and fungal cultures were performed on the blood.

Haematopoietic Recovery
The time of myeloid cell recovery after transplantation was defined as the first of the three consecutive days at which the absolute neutrophils count was at least $0.5x10^9$/L. The time of recovery of platelets was defined as the first seven days on which the platelet count was at least $20x10^9$/L. Engraftment was established by determining the donor-specific short tandem repeats (STR) loci[1,2] in the recipient's bone marrow at day +30, +60 and + 100 post-transplantation.

Graft versus Host Disease (GVHD)
During the first 100 days after transplantation, all patients were evaluated for acute GVHD, which was graded according to the standard practice.

Results

Characteristics of Patients
From September 1999 to October 2001, 10 patients with acute leukemia, chronic myeloid leukemia or aplastic anemia had undergone 11 non-myeloablative PBSC transplantation, in which patient No.1 with CML had graft rejection and was successfully retransplanted later. The patients and disease characteristics are described in Table I. The patients' age ranged between 18 to 49 years (median 33.5 years), male to female ratio is 1:1. Four patients had AML in 1st complete remission. Five patients had chronic myeloid leukemia in chronic phase and 1 patient had severe aplastic anemia. Patients' body weight ranged between 45 to 96 kg (median 58.4kg).

Characteristics of the peripheral blood stem cell allografts
All the patients received allograft from siblings who are HLA matched for HLA-A, HLA-B, HLA-C and HLA-DR. 6 donors were female and 4 were male. All donors received 5 days of subcutaneous Filgrastim 300μg twice daily. All underwent at least 2 apheresis procedures. The CD34+ cell yeild expressed as total number of CD34+ /kg recipient's body weight measured before freezing, ranged between $2.4x10^6$/kg and $14.5x10^6$/kg.

Toxicity
The non-myeloablative PBSC transplantation was better tolerated than the myeloablative protocol. All patients maintained oral intake throughout the procedure and none of them requiring any parenteral nutrition. Febrile episodes were observed in 3 patients, none of them developed veno-occlusive disease (VOD). No mortality was encountered in the first 100 days.

Haematopoietic Recovery
In three patients, their absolute neutrophil counts (ANC) did not fall below $0.5x10^9$/L. The number of days that the ANC was less than 0.5 x 10^9/L in the remaining 7 patients ranged between 5 to 7 days (median, 5 days). Absolute neutrophil count of at least or more than $0.5x10^9$/L was accomplished within 3 to 13 days (median, 9 days). The platelet count was never below $20x10^9$/L in 6 patients and thus requiring no platelet support at all. In the remaining 4 patients, the number of days that the platelet count was less than 20 x 10^9/L ranged between 4 to 7 days. The median time of spontaneous platelet recovery > 20 x 10^9/L was 11.5 days (range, 9 to 13 days). Only 2 out of 10 patients required platelet transfusion once (4 units each). The hospitalization period was also shortened with a median of 14 days (ranged between 13 to 25 days).

GVHD
Acute GVHD developed in 8 paitents while on regular cyclosporin prophylaxis, 5 patients had acute GVHD limited to the skin alone (grade I) and the acute GVHD resolved without further treatment. Patient No.3 and patient No. 7 developed grade III and grade II acute GVHD involving the skin and the liver. Both the patients required additional Prednisolone (2mg/kg/d) therapy. However, when there was intolerance to Cyclosporin and Prednisolone, Mycophenolate Mofetil (MMF) was used as substitute. These two patients subsequently progressed to chronic GVHD. Patient No.8 who had grade I acute GVHD, subsequently progressed to chronic GVHD and was treated with cyclosporin, prednisolone and MMF.

Table I: Patients and disease characteristics

No	Age	Sex	Weight	Diagnosis	Regimen	CD34+ dose (10^6/kg)
1*	30	F	52.5	CML-chronic phase	Fludarabine / cyclophosphamide (Day -3 to -1)	2.6
2	44	F	56.4	AML-M4 1st CR	Fludarabine / cyclophosphamide (Day -3 to -1)	6.8
3	18	M	50	CML-chronic phase	Cyclophosphamide (day -7 to -6) Fludarabine (day -5 to -1)	8.2
4	37	M	68	Aplastic Anemia	Cyclophosphamide (day -7 to -6) Fludarabine (day -5 to -1)	7.8
5	49	F	51.3	AMLL-1st CR	Cyclophosphamide (day -7 to -6) Fludarabine (day -5 to -1)	4.1
6	19	F	59	AML-M4 1st CR	Cyclophosphamide (day -7 to -6) Fludarabine (day -5 to -1)	14.5
7	26	M	57	CML-chronic phase	Cyclophosphamide (day -7 to -6) Fludarabine (day -5 to -1)	9.3
8	48	M	96	AML-M4 1st CR	Cyclophosphamide (day -7 to -6) Fludarabine (day -5 to -1)	3.1
1*	30	F	69	CML-chronic phase Failed 1st transplant For 2nd transplant	Fludarabine (day-7 to -3) Busulfan (day -2 to -1)	3.9
9	47	M	75	CML-chronic phase	Fludarabine (day-7 to -3) Busulfan (day -2 to -1)	4.1
10	39	F	67	CML-chronic phase	Fludarabine (day-7 to -3) Busulfan (day -2 to -1)	2.4

Engraftment

Engraftment was documented in all patients by increasing blood counts and detection of donor-specific STR loci from recipients's bone marrow samples. At day 30 post transplantation mixed chimerism was shown in 6 patients. Patient No. 3 did not attain chimerism at day 30 post-transplantation and was given donor lymphocyte infusion on day 40. Stem cell reinfusion was given on day 140 due to persistent pancytopenia and mixed chimerism. He attained complete chimerism on day 160 and remained so. Patient No. 1 also did not show chimerism at all during the first transplantation but was successfully retransplanted at the 2nd transplantation which showed mixed chimerism on day 30 and until now. Of the remaining 6 patients, Patient No. 6 , No. 4 and No. 7 achieved complete chimerism at day 60, day 100 and day 130 post-transplantation respectively. Patient No.8 remained in mixed chimerism at day 100 post-transplantation. Patient No 2 & 5 relapsed on day 55 and day 60, whilst they were at mixed chimerism state.

Relapse and survival

Until October 2001, with a follow-up period of up to two years (median of 13 months), 8 of 10 patients are still alive and 6 of the 8 alive patients (75%) are disease-free with complete donor chimerism.

Of the 4 patients with acute leukemia, 2 patients relapsed at day 55 and day 60 post-transplantation and died of severe septicemia after reinduction chemotherapy. The other 2 patients are still alive

and well, and in remission at day 296 and day 398 post transplantation.

The 5 patients with CML, 2 patients experienced graft failure on day 100 post transplantation and donor lymphocyte infusions were given, they remained well but both have not attained full donor chimerism. The other 3 patients attained complete chimerism at day 60, day 130 and day 160 post-transplantation and in hematological and molecular remission (bcr-abl regression) at day 70, day 390 and day 495 post-transplantation respectively. However, 2 patients have chronic GVHD involving the skin and mucose membrane and are still on treatment.

The patient with aplastic anemia has attained mixed chimerism at day 60 and subsequently complete chimerism at day 100 post transplantation and remained well until now (day 452 post-transplantation).

Discussion

Our experience shows that the non-myeloablative conditioning regimen for peripheral blood stem cell transplantation (PBSCT) is safe and well tolerated. It appears to be able to secure the engraftment of allogeneic stem cells, while reducing procedure related toxicity substantially.

None of our patients required parenteral nutriti on. We also documented few febrile episodes due to intercurrent infections, no or short period of neutropenia (the median duration for ANC > $0.5x10^9$/L is 10 days, ranged: 3 to 13 days), no or short period of thrombocytopenia (the median duration for platelet > $20x10^9$/L is 11.5 days, ranged : 9 to 13 days) with low incidence of platelet dependency. Slavin et al[3] in 1998 had documented that ANC > $0.5x10^9$/L was accomplished within 10-32 days (median 15 days) and spontaneous platelet counts > $20x10^9$/L were achieved within 0 - 35 days (median 12 days). Child et al[4] in 1999 had similarly demonstrated that a faster neutrophil recovery to > 500/μl at a median of 11 days (range, 9 - 15 days) and the median time to a platelet count >50,000/μl was 8 days (range,0 - 10 days). Haemopoietic recovery is much faster as compared to allogeneic PBSCT using the myeloablative conditioning protocol which showed a median time to achieve a neutrophil count of > $0.5X10^9$/L and a platelet count of > $20x10^9$/L ranged from 14 to 15 days and 14 to 16 days respectively,[5,6,7,8]. The median blood support required in myeloablative allogeneic PBSCT recipients was documented to be 8 units of red blood cells and 24 units of platelets[9]. In our patients, only patient No 4 (severe aplastic anemia) required 2 units of packed red cells and 8 units of random donor platelets during the 1st 30 days post transplantation. None of our patients experienced veno-occlusive disease of the liver or other complication such as interstitial pneumonitis or multiorgan failure. These findings were comparable with Childs et al[4] who had only demonstrated 1 case with mild VOD and 1 case with bilateral pulmonary infiltrates. No transplant-related mortality was documented. Besides, the hospitalization period was also shortened to a median of 17 days. With this shortened hospital stay and fewer complications, the cost of NMT was estimated to be lower than that of the myeloablative transplantation.

In elderly individuals, who normally are not eligible for a myeloablative allogeneic transplant, non-myeloablative protocol may permit a relatively safe clinical application of a potentially curative procedure based primarily on adoptive immunotherapy and thus improve treatment outcome in them.

However, severe GVHD remains an important cause of morbidity. Previous authors [5,6,7,8] have observed an incidence of acute GVHD after allogeneic standard transplantation ranged from 38% to 66% (grade II to IV) and 13% to 25% (grade III to IV), whereas the incidence of chronic GVHD observed ranged between 32% to 79%. Slavin et al[3] and Child et al[4] have reported the occurrence of severe acute GVHD after peripheral blood stem cell transplantation of 25% and 60% respectively.

The incidence of chronic GVHD was only 36% and 27% respectively and was mild and limited. In our series, the grade I acute GVHD was observed in 60% of the patients, majority were very mild. Only two patients (20%) developed severe grade III acute GVHD which subsequently progress to chronic GVHD who required prolonged medications.

Controversy exists as to whether the incidence of GVHD is increased after PBSCT when compared with bone marrow transplantation (BMT). This is because PBSCT typically transferred a significantly larger dose of mature, immunocompetent T cell of the donor origin to the recipient. A meta-analysis on GVHD was reported by Cutler et al[10] recently showing that the pooled relative risk (RR) for acute and chronic GVHD after PBSCT was 1.16 (95% confidence interval, 1.04 - 1.28; P=0.006) and 1.53 (95% CI, 1.25 - 1.88; P < 0.001) respectively when compared with traditional BMT. He also reported a RR of developing clinically extensive chronic GVHD was 1.66 (95% CI, 1.35 - 2.05; P<0.001). A meta-regression model has demonstrated that the RR of GVHD increased as the difference in T cells delivered between the PBSCT and BMT grafts increased, i.e once the differences in T cells transferred was controlled for, there was a trend that showed peripheral blood stem cells to be protective against the development of chronic GVHD. However, the risk of malignant relapse after PBSCT was lower than after BMT. Therefore, with the introduction of new technology to improve the composition of the graft by optimizing the number of reactive T-cells or alternatively by improvement of GVHD prophylaxis and treatment, the incidences of severe GVHD may be reduced in PBSCT while maintaining a lower rate of relapse.

Conclusion

Our series indicates that non-myeloablative PBSCT is associated with a faster engraftment, few or no blood components required, a shortened hospital stay, no greater incidence of severe acute or chronic GVHD and an overall reduction in total cost.

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