

Graft-Versus-Host Disease after Allogeneic Hematopoietic Stem Cell Transplantation with Non-Myeloablative Conditioning: Experiences at a Single Center

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Background: The use of non-myeloablative stem cell transplantation (NST) has recently been increasing for treating the patients who cannot tolerate ablative hematopoietic stem cell transplantation (HSCT). Although graft-versus-host disease (GVHD) is one of the greatest problems in HSCT, the clinical effect of GVHD following NST is not clear. We undertook this study to evaluate the clinical manifestations of GVHD and the outcomes after NST.

Methods: From October 2000 to October 2004, 61 patients underwent NST with a fludarabine-based conditioning regimen. The cumulative incidence of GVHD and the survival rates were obtained from the Kaplan-Meier curves.

Results: With a median follow-up of 195 days, the estimate for overall three-year survival was 32%. The cumulative incidences of grades II~IV acute GVHD and chronic GVHD were 33% (18/53) and 78% (29/37), respectively. The response rates for acute and chronic GVHD were 33% and 89%, respectively. The survival rates of patients with acute and chronic GVHD were 27% and 89%, respectively. The median survival time was 6.5 months

Conclusion: The incidence of GVHD after NST did not differ from that after ablative HSCT. This study suggests that the aggressive treatment of acute GVHD should be considered to improve the overall survival after NST. (*Korean J Hematol 2006;41:92-98.*)

Key Words: Hematopoietic stem cell, Non-myeloablative transplantation, Graft-versus-host disease, Preparative regimen

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INTRODUCTION

Ablative allogeneic hematopoietic stem cell transplantation (HSCT) for the treatment of hematological malignancies originally depended on the effect of myeloablative cytotoxic chemoradiotherapy. However, allogeneic stem cells were found to exert lethal immunological effects on tumor cells, called the graft-versus-tumor (GVT) effect.¹⁻⁴⁾

Recently, a new strategy for allogeneic stem cell transplantation using reduced-intensity or non-myeloablative conditioning has been developed to reduce regimen-related toxicity while exploiting GVT effects.³⁻⁷⁾ This prevents major regimen-associated complications, making it possible to treat older and medically infirm patients who are at high risk of treatment-related complications after conventional conditioning.

Graft-versus-host disease (GVHD) has long been recognized as a serious and frequent complication of conventional ablative HSCT. GVHD has remained a major determinant of posttransplantation morbidity, quality of life, and survival. Previous studies have reported the clinical features of GVHD, based on experience over decades of conventional ablative allogeneic HSCT.

Several aspects must be considered to understand the clinical manifestations of GVHD following non-myeloablative stem cell transplantation (NST), compared with those after conventional ablative HSCT.⁸⁻¹¹⁾ First, patients who receive non-myeloablative conditioning are usually older than patients undergoing a myeloablative regimen, and increasing age is associated with an increasing risk of GVHD.¹²⁾ Second, because gastrointestinal damage appears to play a role in the initiation of GVHD, it is not unexpected that the decreased toxicity involved in the conditioning for NST decreases the incidence of GVHD below that observed with myeloablative HSCT. Third, donor lymphocyte infusions intended to achieve GVT with the introduction of donor stem cell

chimerism enhance the severity and incidence of GVHD.¹³⁾ Although the immunobiology of NST differs from that of ablative HSCT, the clinical manifestations of GVHD following NST are not known in detail.

In this study, we retrospectively analyzed data from 61 patients who had undergone NST, to determine the incidence, severity, timing, and clinical outcomes of GVHD after NST.

MATERIALS AND METHODS

1. Patients

Sixty-one patients who had undergone NST at the Seoul National University Hospital between October 2000 and October 2004 were included in this study. Their detailed characteristics are listed in Table 1. Men (52 patients) were more common than women (nine patients). Their pretransplantation characteristics are summarized in Table 2. Most of the 61 patients (52 patients) had hematological malignancies. Thirty-three (54%) patients received NST in refractory status. Sixteen (26%) patients who could not receive high-dose chemotherapy due to old age or

Table 1. Patient characteristics

Characteristic	n (%)
Age (years)	
Median	48
Range	17~67
Sex	
Male	52 (85%)
Female	9 (15%)
Donor	
Related	50 (81%)
Unrelated	11 (19%)
Stem cell source	
Peripheral blood stem cell	51 (83%)
Bone marrow	2 (3%)
Both	8 (13%)
Conditioning	
Fludarabine/melphalan	38 (62%)
Fludarabine/cyclophosphamide	23 (38%)

Table 2. Diseases of patients who received NST

Pretransplantation disease	Total number	Relapse/refractory (n)
Acute myelogenous leukemia	20	5/11
Acute lymphoblastic leukemia	10	7/3
Non-Hodgkin's lymphoma	8	0/8
Multiple myeloma	4	0/4
Chronic myelogenous leukemia	2	0/2
Mylelodysplastic syndrome	8	-
Renal cell carcinoma	5	0/5
Aplastic anemia	4	4/0

combined morbidity underwent NST.

All but one patient receiving HSCT did so from a related or unrelated donor who was serologically matched for HLA-A, -B, -C, and -DR. Unrelated donor transplantations were performed in 11 patients, including one patient with one mismatched locus. Most patients received peripheral blood, but two patients received bone marrow only, and eight patients received both peripheral blood and bone marrow as sources of stem cells.

2. Conditioning regimens and postgrafting immunosuppression

Patients received chemotherapy based on fludarabine (30mg/m² for five consecutive days), with melphalan (90mg/m² for two consecutive days) for myeloid malignancies or cyclophosphamide (60mg/m² for two consecutive days) for lymphoid or renal cell cancer. All patients were given cyclosporine (3mg/kg i.v. daily for 28 days, starting one day before NST) as postgrafting immunosuppression. If bone-marrow analysis 28 days after NST showed full donor chimerism, the cyclosporine regimen was changed to an equivalent dose given in an oral form until day 60 after NST. The cyclosporine dose was then tapered until day 100, in the absence of GVHD. Without complete chimerism or overt GVHD, cyclosporine was tapered rapidly over two weeks.

3. GVHD grading and treatment

Diagnosis and clinical grading of acute and chronic GVHD were performed according to established criteria.^{14,15)} Treatment was given for grades II~IV acute GVHD and extensive chronic GVHD. Initial treatment usually consisted of prednisolone (1~2mg/kg daily) for 14 days and then tapered. In addition, the administration of cyclosporine, mycophenolate mofetil (15mg/kg orally every 12hours), or both were usually resumed at full doses. Extensive chronic GVHD were usually treated with prednisolone and mycophenolate mofetil with or without alternate-day cyclosporine by attending physician's assessment.

4. Infection prophylaxis and supportive care

All patients were given fluconazole (100mg, orally daily) and ciprofloxacin (500mg, orally twice a day) for three consecutive days, starting on day 6 before NST, for gastrointestinal decontamination. Prophylaxis against *Pneumocystis carinii* was undertaken with trimethoprim-sulfamethazole, starting on day 20 after conditioning, until the absolute neutrophil count (ANC) increased to more than $0.5 \times 10^9/L$. When cytomegalovirus (CMV)-negative patients received a transplant from a CMV-seropositive donor, PCR surveillance of blood samples for CMV and urine CMV cultures were performed every two weeks to allow preemptive acyclovir treatment. Granulocyte-colony-stimulating factor treatment was commenced on the day of conditioning until an ANC of more than $1.0 \times 10^9/L$ was achieved. Prophylactic platelet transfusions were performed when platelet counts dropped below $10 \times 10^9/L$. Granulocyte transfusions were performed in patients with neutropenic fever refractory to antibiotics and antifungal agents.

5. Chimeric status evaluation for donor lymphocyte infusion (DLI)

After NST, chimerism and clonal disease mar-

kers must be closely monitored for the early detection of graft failure or relapse. We assessed chimeric status by variable number tandem repeats (VNTR) analysis and fluorescence in situ hybridization analysis of bone marrow on days 14 and 28, and of peripheral blood on days 60, 90, 120, and 150.

DLI was performed to achieve full donor chimerism and GVT in patients without GVHD who had discontinued cyclosporine treatment. The alloreactive T cell subset (CD3) used for DLI was increased to 5×10^7 /kg until the development of complete chimerism, GVHD, or disease regression.

6. Statistical analysis

Survival curves and the cumulative incidence of GVHD were evaluated using the method of Kaplan and Meier; 95% confidence intervals were calculated. Cumulative incidence curves were calculated for grades II~IV acute GVHD and extensive chronic GVHD. The statistical analysis was performed using the SPSS 11.0 software package (SPSS Inc., Chicago, IL, USA)

RESULTS

1. Outcomes of NST

Thirteen patients died within 30 days after NST. Among these patients, early treatment-related death, which was evaluated 30 days after NST, was observed in 6 (10%) patients. The commonest cause of death was infection (4 patients). Two patients died of myocardial infar-

Table 3. Outcome of NST

Pretransplantation disease	Evaluated number	n (%)
Chimerism	53	42 (80)
Response	50	20 (40)
Early Mortality	61	13 (21)
Survival Rate*	61	33%

*Three-year survival rate.

tion or central nervous system hemorrhage.

The median ANC nadir was 0.2×10^9 /L and ANC was below 0.5×10^9 /L for a median 11 days. Twelve patients had no engraftment. Among 53 patients who could be evaluated, complete and mixed chimerism was observed on day 14 in 36 and 6 patients, respectively. Responses were evaluated in 50 patients 90 days after NST. Complete or partial remission was observed in 20 patients.

The three-year survival rate and the median survival time of the 61 patients were 32% and 6.5 months (95% CI, 2~11 months), respectively (Fig. 1, Table 3).

2. Acute GVHD

Acute GVHD could not be evaluated in eight patients because of their early deaths. The cumulative incidence of grades II~IV acute GVHD was 33% (18/53) (Fig. 2A). Grades II, III and IV GVHD were observed in four, six, and eight patients, respectively. Of the 61 patients, 8 patients died before the development of acute GVHD. In 18 patients who developed acute GVHD (33%), 6 patients (33%) responded to treatment. The median time to the initiation of corticosteroid treatment was 32 days.

Of 18 patients with grades II~IV acute GVHD, the earliest onset of acute GVHD was 7 days and the latest was 88 days after NST. The

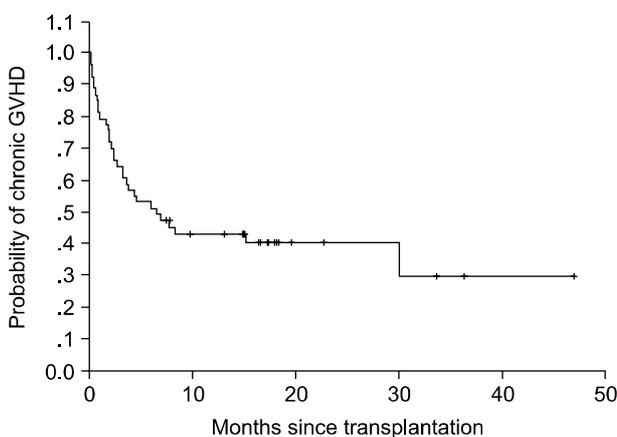


Fig. 1. Kaplan-Meier survival curve of overall survival after NST.

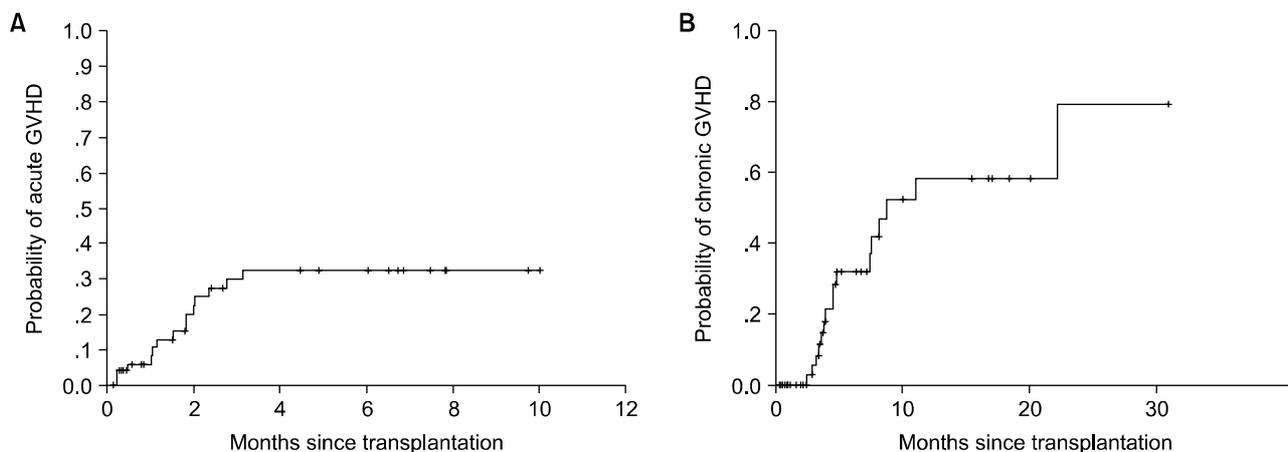


Fig. 2. Cumulative incidence of grade II~IV acute GVHD (A) and chronic GVHD (B).

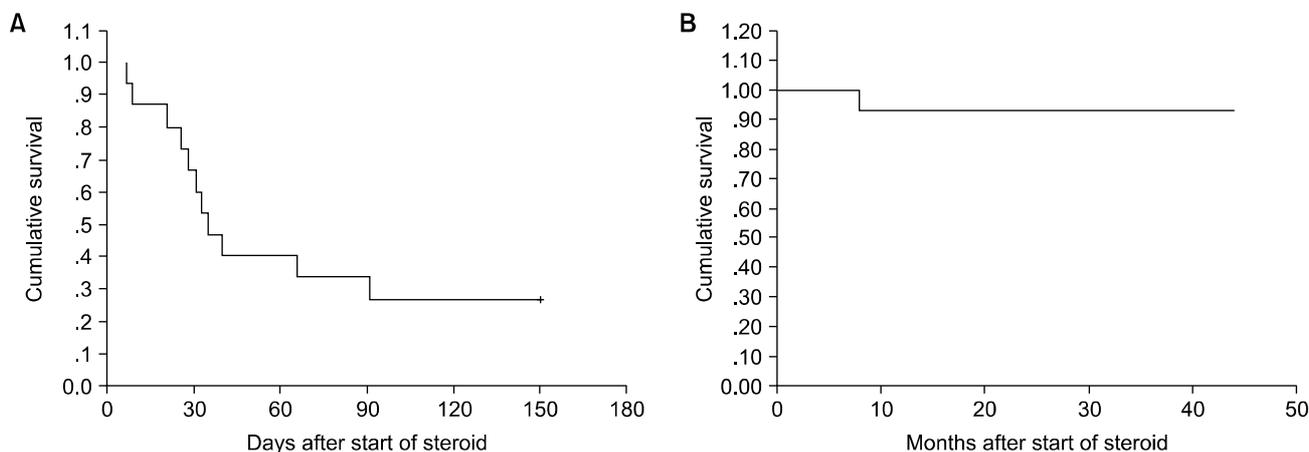


Fig. 3. Survival curves of acute (A) and chronic (B) GVHD under immunosuppressive treatment.

five-month survival rate of patients treated for acute GVHD was 27% (Fig. 3A).

3. Chronic GVHD

Thirty-seven patients lived for over 70 days after NST. Of these, 29 patients manifested chronic GVHD, 17 extensive GVHD (45%; 17/37) and 12 limited GVHD. In 12 patients, chronic GVHD was preceded by acute GVHD, either the resolved or unresolved form. Seventeen patients with chronic GVHD experienced no prior acute GVHD; this is de novo onset chronic GVHD.

The cumulative incidence of chronic GVHD was 78% (29/37) (Fig. 2B). The median time to the initiation of treatment for chronic GVHD was 125 day. The response to treatment of patients

with chronic GVHD was 89% (26/29). The three-year survival rate of patients with chronic GVHD undergoing treatment was 89% (Fig. 3B).

DISCUSSION

The incidence of acute GVHD is lower after NST than after myeloablative HSCT, but this is not true of chronic GVHD.¹⁶⁻¹⁸⁾ However, other data have shown a similar incidence of acute GVHD after both NST and myeloablative HSCT in old age.¹⁹⁾

Ethnicity might influence the risk of GVHD, with Japanese patients having a lower incidence of GVHD than western patients.²⁰⁾ However, our study showed that the incidence of grades II~IV

acute GVHD (33%) and extensive chronic GVHD (45%) was not lower than that of myeloablative HSCT performed in Korea or Japan.^{20,21)} The use of peripheral blood as the source of hematopoietic stem cells is another cause of the high incidence of GVHD. These findings are, in part, consistent with the results of another study²²⁾ that reported a high incidence of grades II~III GVHD (82.8%) and chronic GVHD (78.6%) when peripheral blood was used as the source of hematopoietic stem cells.

Acute GVHD was treated with immunosuppressants such as corticosteroid, cyclosporine, and mycophenolate mofetil. It is generally known that the commencement of treatment for GVHD is delayed after NST, compared with treatment after myeloablative HSCT, because of the lower incidence of GVHD associated with NST.¹⁶⁾ In our study, the median time to the initiation of corticosteroid treatment was 33 days. This suggests that the onset of acute GVHD was not delayed relative to that after myeloablative HSCT. Furthermore, the response rate and survival outcome with manifestations of acute GVHD in receipt of treatment (27%) were lower than those reported in earlier studies performed in Korea.²¹⁾

Unlike patients with acute GVHD, those with chronic GVHD responded relatively well to immunosuppressive therapies such as corticosteroid, cyclosporine, mycophenolate mofetil, hydroxychloroquine, and anti-thymocyte globulin (26/29; 89%).

Overall survival at one year (42%) and three years (32%) was lower than that observed after other myeloablative HSCT trials performed in Korea and Japan.²⁰⁻²²⁾ The causes of the low overall survival seem to have been the high incidence of GVHD and early mortality.

In conclusion, the incidence of GVHD after NST did not differ from that after myeloablative HSCT performed in Korea or Japan. Patients with acute GVHD had poor survival outcomes compared with those of patients with chronic

GVHD. This study suggests that acute GVHD should be treated aggressively to improve the outcomes of NST.

요 약

배경: 비골수억제성 조혈모세포이식술(non-myeloablative hematopoietic stem cell transplantation, NST)은 전통적인 조혈모세포 이식술에 적합하지 않은 환자에서 최근 많이 시행되는 추세를 보이고 있다. 이식편대 숙주반응은 조혈모세포 이식술 후 발생하는 심각한 합병증 중의 하나이지만, NST시행 후 발생한 이식편대 숙주반응은 임상상 및 예후가 잘 알려져 있지 않다. 본 연구는 NST 후 발생한 이식편대 숙주반응의 임상상 및 예후에 관한 후향적 연구이다.

방법: 2000년 10월부터 2004년 10월까지 NST를 시행한 61명에 대해서 급성 및 만성 GVHD의 발생률 및 치료효과와 생존율에 대해서 후향성 조사를 하였다. 발생률 및 생존율은 카플란-마이어 생존 곡선으로 구하였다.

결과: 전체 환자의 중앙 생존 기간은 6.5개월이었다. II도 이상의 급성 및 만성 이식편대 숙주반응의 발생률은 각각 33% (18/53)와 78% (29/37)였으며, 치료율은 각각 33% (6/18)와 89% (26/29)였다. 급성 및 만성 이식편대 숙주반응으로 치료를 받은 환자의 생존율은 27%와 89%였다.

결론: NST 후 발생하는 이식편대 숙주반응은 전통적인 조혈모세포이식에 비해 이식 편대 숙주반응의 빈도가 유사한 것으로 보인다. NST의 성적을 향상시키기 위해 급성 이식편대 숙주반응은 적극적인 치료가 필요할 것으로 판단된다.

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