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Correlation Between Survival and Number of Mobilized CD34+ Cells in Patients with Multiple Myeloma or Waldenström Macroglobulinemia

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Abstract High-dose chemotherapy followed by autologous stem cell transplantation is the established treatment for symptomatic multiple myeloma (MM) or Waldenström macroglobulinemia (WM). We retrospectively analyzed the impact of mobilized CD34+ cell number on clinical outcomes in patients with MM or WM who underwent autologous stem cell transplantation in our hospital from 1997 to 2007. A total of 39 patients were identified. All patients received peripheral stem cell support after a conditioning regimen. We defined patients with collection of a large number ($\geq 8\times10^6$/kg) of CD34+ cells as super mobilizers (SM), and all others as normal mobilizers (NM). Although hematological engraftment was earlier in the SM group, overall survival did not differ significantly between groups (P=0.392). Likewise, no significant differences were seen in progression-free survival (P=0.201) or survival after relapse (P=0.330). In conclusion, our retrospective study could not find any correlation between survival and number of mobilized CD34+ cells, in contrast to previously reported results.

Keywords Mobilized CD34+ cell number · Multiple myeloma · Waldenström macroglobulinemia · Autologous stem cell transplantation

Introduction

High-dose chemotherapy followed by autologous stem cell transplantation (HDC-ASCT) is now widely performed for many hematological malignancies, including malignant lymphoma, acute leukemia, Waldenström macroglobulinemia (WM), and multiple myeloma (MM). In MM, HDC-ASCT shows higher rates of complete response (CR), progression-free survival (PFS) [1–4], and, in some studies, increased overall survival (OS) compared with conventional chemotherapy [1–3]. Improvements in outcomes during the last 10 years are associated with greater proportions of patients achieving major responses to HDC-ASCT. After the advent of new agents such as bortezomib and thalidomide, the therapeutic strategy for MM is changing rapidly. However, HDC-ASCT is still considered the mainstay therapy for MM, particularly in younger patients. To clarify the patient subsets that will benefit most from HDC-ASCT, some predictive factors have been reported [5–14]. Of these, the number of mobilized or infused CD34+ cells has been reported to influence outcomes after ASCT in hematological malignancies [7, 9, 10, 13]. However, these results were conflicted in part and only limited reports have correlated the number of CD34+ cells with clinical outcomes in patients with MM or WM [9, 13]. This issue thus remains controversial. To assess the impact of mobilized CD34+ cell number on survival after HDC-ASCT in patients with MM or WM, we retrospectively analyzed 39 patients who underwent HDC-ASCT in our hospital and investigated correlations between mobilized CD34+ cell number and clinical outcomes.

Patients and Methods

Subjects comprised all 39 patients (19 men, 20 women) who were diagnosed with either MM or WM and received HDT-ASCT from June 1997 to May 2007 in our hospital. No patients were excluded. The median age of patients was

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Table 1 Patients characteristics

Patient, n		Total 39	NM 22	SM 17	P value
Age, median (range)		59 (33–69)	61 (33–65)	58 (51–69)	0.542
Gender, n	Male	19	8	11	0.079
	Female	20	14	6	
Diagnosis	MM	37	21	16	
	WM	2	1	1	
Mobilizing regimens	VP-16 + GCSF	30	15	15	0.251
	CY or DCEP + GCSF, or GCSF only	9	7	2	
No. of prior chemotherapies, mean (range)		3.69 (0–15)	4.32 (2–15)	3.12(0–5)	0.834
No. of mobilized CD 34 cells (×106/kg), mean (range)		10.7638 (1.0–50.16)	3.9473 (1–7.91)	19.5853 (9.73–50.16)	
No. of apheresis, mean (range)		1.56 (1–6)	2 (1–6)	1 (1)	0.003
Disease status at 1st SCT	CR or PR	24	11	13	0.051
	>MR, or relapse	15	11	4	
No. of SCT, mean (range)		1.54 (1–3)	1.55 (1-2)	1.53 (1–3)	0.117
Conditioning regimens	L-PAM	34	17	17	
	L-PAM + TBI	3	3	0	
	L-PAM + VP-16 + MCNU	1	1	0	
	L-PAM (1st) → Cy + TBI (2nd)	1	1	0	
Duration from diagnosis to 1st SCT, median months (range)		11 (4–126)	13.5 (8–87)	11 (4–126)	0.001
Duration from diagnosis to 2nd SCT, n, median months (range)		*n*=17, 18 (11–130)	*n*=10, 22.5 (16–39)	*n*=7, 16 (11–130)	0.260
Infused CD 34 cell dose (×106/kg), mean,(range)		3.52 (1–12.6)	1.89 (1–3.31)	5.18 (2.43–12.6)	<0.001

NM normal mobilizers, *SM* super mobilizers, *MM* multiple myeloma, *WM* Waldentröm's macroglobulinemia, *CY* cyclophosphamide, *GCSF* granulocyte colony stimulating factor, *DCEP* dexamethasone, cyclophosphamide, etoposide and cisplatin, *CR* complete response, *PR* partial response, *MR* minor response, *SCT* stem cell transplantation

59 (range, 33–69 years). All patients received autologous peripheral blood stem cell (PBSC) transplantation. For most (n=30), PBSCs were collected with etoposide plus granulocyte colony-stimulating factor (G-CSF), with the remainder collected with cyclophosphamide (Cy) plus G-CSF (n=6), combination chemotherapy with dexamethasone, Cy, etoposide, cisplatin (DCEP regimen) plus G-CSF ≥ (n=2), or G-CSF only (n=1). According to a previous study [7], patients were defined as super mobilizers (SM) if numbers of mobilized CD34+ cells were ≥ 8×10^6/kg, and as normal mobilizers (NM) if $<8\times10^6$/kg. In these two groups, we retrospectively analyzed the impact of the number of mobilized CD34+ cells on OS and PFS. Among patients displaying disease progression, we also evaluated survival after disease progression. Disease progression was defined as any increment in serum M-protein, or increment of Bence-Jones proteinuria or other manifestations of myeloma such as hypercalcemia, bone lesion, or soft tissue plasmacytoma.

Continuous baseline characteristics were compared between SM and NM using the Mann-Whitney test. Categorical characteristics were compared using the χ^2 test or Fisher's exact test if necessary. OS was calculated from first transplant to final follow-up or death. PFS was calculated from first transplant to final follow-up or the

Table 2 Hematopoietic recover and neutropenic fever

	NM n=22	SM n=17	P value
Neutrophil recovery (> 500/μl), Mean days (range)	11 (9–13)	10 (9–12)	0.012
Platelet recovery (> 50,000/μl), Mean days (range)	17 (12–42)	13 (10–15)	0.004
No. of episode of neutropenic fever	16	11	0.685
Duration, mean days (range)	3 (1–7)	2 (1–5)	0.200

NM normal mobilizers, *SM* super mobilizers

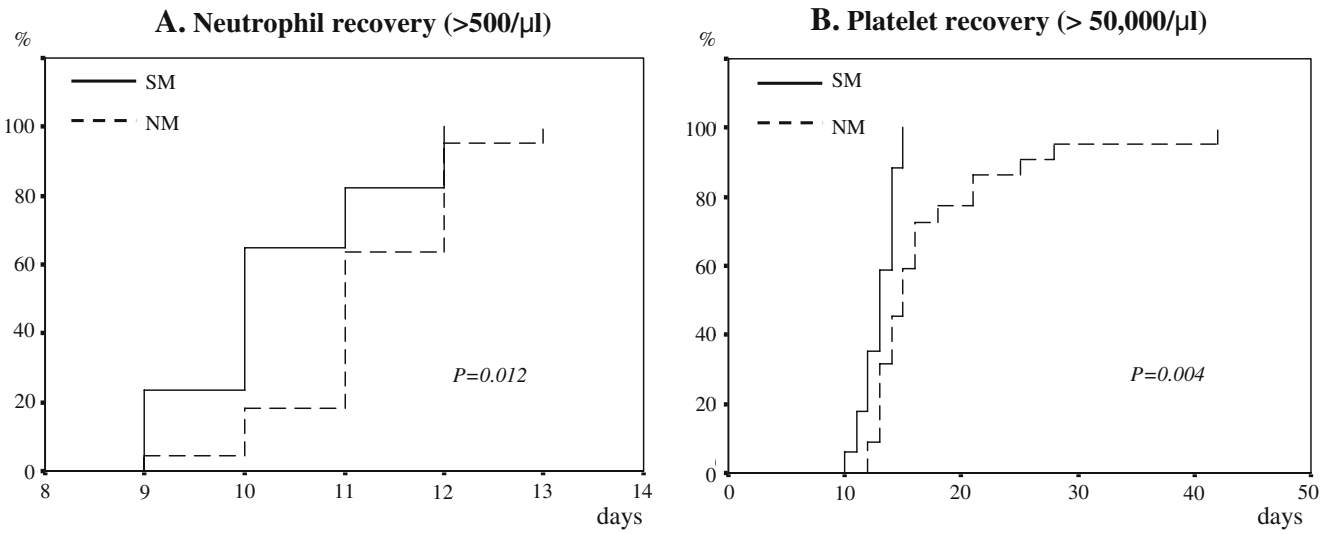

Fig. 1 **a** Neutrophil recovery to >500/µl and **b** platelet recovery to > 50,000/µl. Abbreviations: NM, normal mobilizers; SM, super mobilizers

date on which disease progression was first observed. Survival after disease progression was calculated from the date of disease progression to final follow-up or death. Outcomes were estimated using Kaplan-Meier methods and compared between mobilized groups using the log-rank test. All analyses were performed using SPSS II software (SPSS, Chicago, IL). All statistical tests were two-sided, and values of $P<0.05$ was considered statistically significant.

Results

The 39 patients included 37 patients with MM and 2 patients with WM. Seventeen patients were identified as SM, and 22 as NM (Table 1). SM and NM patients did not differ significantly in terms of age, gender, mobilizing regimens, number of prior chemotherapies, number of transplantations, or disease status at first transplantation. As expected, median infused CD 34+ cell doses were significantly higher in the SM group (5.18×10^6 CD34 cells/kg in SM, 1.89×10^6 CD34 cells/kg in NM; $P<0.001$). Number of apheresis and duration from diagnosis to first transplantation were also significantly different between groups (Table 1).

All patients received melphalan-based conditioning chemotherapy, with 34 patients receiving melphalan only, 3 patients receiving melphalan plus total body irradiation (TBI), 1 patient receiving melphalan combined with etoposide and ranimustine, and 1 patient treated with melphalan at first transplantation and Cy plus TBI at the second transplantation. All patients achieved engraftment and no treatment-related deaths were observed. SM patients showed more rapid engraftment of neutrophils and platelets (Table 2). Median duration to neutrophil recovery (neutrophils>500/µl) was 10 days for the SM group, compared to 11 days for the NM group ($P=0.012$, Fig. 1a). Median duration to platelet recovery (platelets > 50,000/µl) was 13 days in the SM group, compared to 15 days in the NM group ($P=0.004$, Fig. 1b). No significant difference was seen in the incidence of febrile neutropenia ($n=16$ in SM, $n=11$ in NM, $P=0.685$, Table 2). Non-hematological, grade3 or 4 regimen related toxicities (RRTs) were listed on Table 3. The incidence of RRTs was not statistically significant and total events were similar between groups (20 in NM, 19 in SM).

The median follow-up period was 38 months (range 3 to 93 months). No significant differences in OS ($P=0.392$, Fig. 2) or PFS ($P=0.201$, Fig. 3a) were apparent between groups. Disease progression was seen in 23 patients (59%) from the entire group, comprising 7 SM patients and 16 NM patients. Although NM patients tended to show a higher rate of disease progression than SM patients, no significant difference was apparent (NM, 72%; SM, 41%, $P=0.098$) and survival after disease progression also did not differ significantly between groups ($P=0.330$, Fig. 3b).

Table 3 Non-hematological regimen related toxicities (grade 3 or 4)

	Total	NM	SM	P value
Patients, n	27	17	10	0.222
Stomatitis	10	6	4	
Nausea	8	6	2	
Diarrhea	7	2	5	
Engraftment syndrome	3	1	2	
Skin rash	2	1	1	
Others	9	4	5	
Total events	39	20	19	

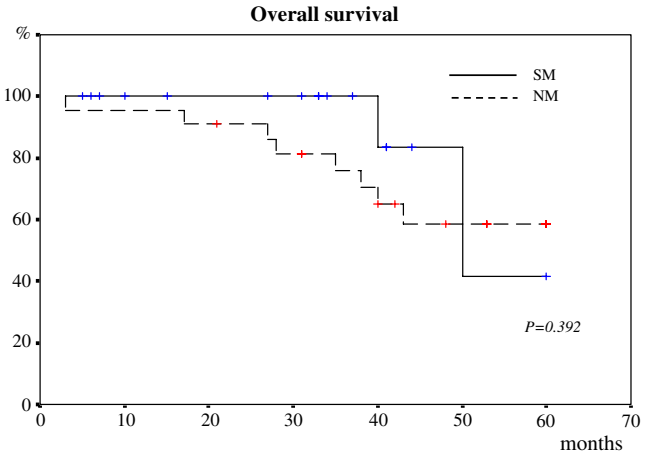

Fig. 2 Overall survival. Abbreviations: NM, normal mobilizers; SM, super mobilizers

Discussion

High-dose melphalan followed by ASCT is now widely used as the frontline therapy in younger patients with MM and WM. Some clinical characteristics have been reported as predictive factors for survival following ASCT, including numbers of both mobilized and infused CD34+ cells [7, 9, 10, 13].

In patients with malignant lymphoma, poor mobilizers have been associated with inferior outcomes, whereas SM have shown better survival after relapse and overall better OS [7, 10]. The present study, however, could not find any significant correlation between OS and the number of mobilized CD34+ cells in patients with MM or WM (Fig. 2). Survival after disease progression was also similar between groups (Fig. 3b). Our results also contrast with a report by Eriksson *et al.*, who found that the total number of mobilized CD34+ cells was significantly associated with OS in patients with MM, according to multivariate analysis [13]. In that study, CD34+ cells were harvested much more in patients who achieved CR or partial response at transplantation than in patients who achieved minor response or no response. Likewise, in a study of malignant lymphoma [7], patients in the SM group were younger and had received fewer prior chemotherapies than those in the NM group, suggesting that patients from whom a larger number of CD34+ cells were harvested might have included more patients with relatively favorable underlying disease in both studies. Our patients were well balanced for age, gender, mobilizing regimens, number of prior therapies, number of transplantations, and disease status at transplantation (Table 1). The present results might thus better reflect the intrinsic impact on patient survival. Furthermore, some studies have failed to show any correlation between CD34+ cell number and patient survival, which would also support the reliability of our results [5, 9].

The possibility remains that a larger number of infused CD34+ cells causes rapid hematological recovery, decreased likelihood of complications associated with cytopenia, and better outcomes. As previously reported [7, 8], earlier neutrophil and platelet recoveries were observed in the SM group, but although these differences were statistically significant, the actual clinical significance remains unclear. Neutrophil recovery (> 500/μl) occurred 1 day earlier and platelet recovery (> 50,000/μl) occurred 2 days earlier in the SM group (Table 2, Fig. 1), but had no major impact on survival under appropriate supportive care. Incidence of febrile neutropenia was similar in both groups (Table 2). Moreover, non-hematological, grade 3 or 4 RRTs were also similar (Table 3) and no treatment-related deaths were observed in our study. The composition of harvested cells is also known to affect clinical outcomes in ASCT. Increasing dendritic cell count in autologous stem cell graft

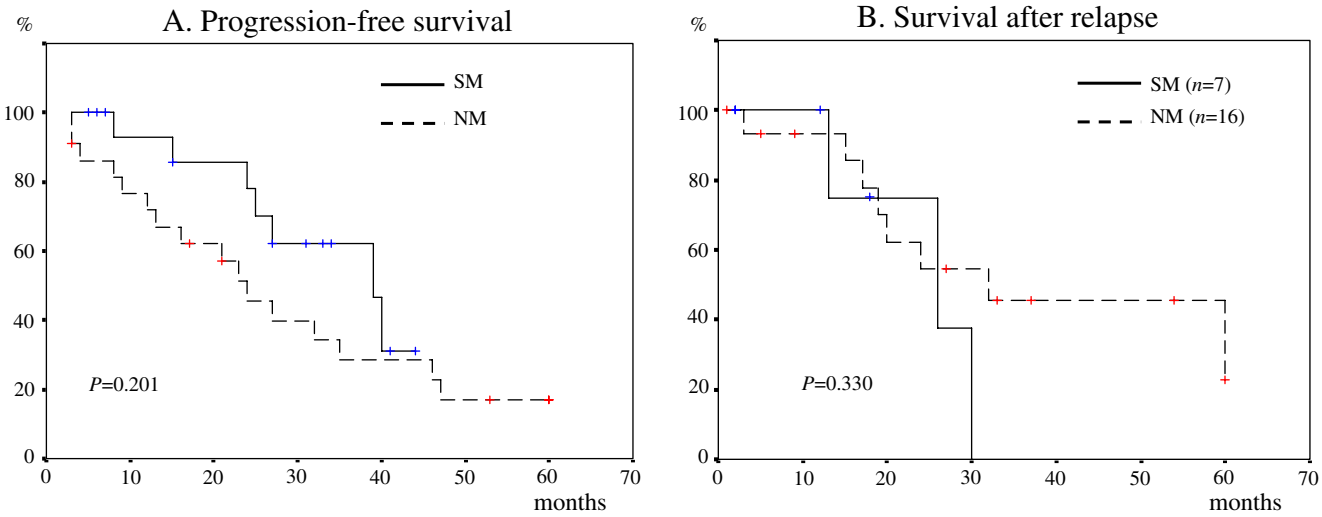

Fig. 3 **a** Progression-free survival **a** and **b** survival after relapse. Abbreviations: NM, normal mobilizers; SM, super mobilizers

has been associated with improved survival in ASCT for diffuse large B-cell lymphoma [15]. A recent study found that a higher infused lymphocyte dose predicts superior OS following ASCT for MM [6]. Although stem cell grafts from SM may contain a larger number of dendritic cells and/or lymphocytes than those from NM, we were unable to investigate these issues in the present retrospective study. Further evaluation of these questions is thus required.

In summary, we analyzed correlations between the number of mobilized CD34+ cells and clinical outcomes in patients with MM or WM. Our data have demonstrated no significant differences in patient survivals between two groups. In view of the retrospective nature, the relatively small number of patients, and lack of multivariate analysis in our study, further investigation will be necessary to confirm these correlations in analyses of large, well-balanced patient populations.

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