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ORIGINAL ARTICLE

Mobilization of PBSCs with chemotherapy and recombinant human G-CSF: a randomized evaluation of early vs late administration of recombinant human G-CSF

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The optimal timing for recombinant human (rh)G-CSF administration after chemotherapy for PBSC mobilization has not yet been determined. In this study, we compared two different time schedules of rhG-CSF; 4th (early) vs 7th day (late), in 48 consecutive patients with multiple myeloma and lymphoma undergoing PBSC mobilization with CE (CY 4 g/m^2 on day 1 and etoposide 200 mg/m^2 on days 1–3). The rhG-CSF dose was 10 μg/kg/day for all patients. Both groups were comparable in terms of sex, age and number of previously given different chemotherapy regimens. Duration of neutropenia, CD34$^+$ cell count on the first day of apheresis and numbers of aphereses were not statistically different between the two arms. However, the number of doses of rhG-CSF up to the first cycle of apheresis procedures was significantly lower in the late group than in the early group ($P = 0.005$). The median number of total CD34$^+$ cells collected was 10.54×10^6/kg (range 0.11–37.27) in the early group and 10.81×10^6/kg (range 0.17–49.83) in the late group of rhG-CSF ($P = 0.781$). We conclude that PBSC mobilization after late use of rhG-CSF is an effective approach and therefore, in routine clinical practice, late rhG-CSF may be used for PBSC collections after chemotherapy-based mobilization regimens in this cost-conscious era.

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Introduction

PBSCs are currently the preferred source of stem cells for autologous transplantation after administration of high-dose chemotherapy.[1] Published studies suggest that fairly intensive chemotherapy in combination with hemopoietic growth factors results in the collection of more progenitor cells, and that adequate collection correlates with several factors as reported earlier.[2–9] Growth factors alone or in combination with chemotherapy have been used in daily doses of 5–40 μg/kg, and recombinant human (rh)G-CSF at a dose of 10 μg is the most commonly used and recommended dose.[10–13] However, the optimal timing for rhG-CSF initiation after chemotherapy has not been determined as conclusively as has the rhG-CSF dose.

We have previously reported the influence of different doses of rhG-CSF on stem cell yield using chemotherapy and rhG-CSF-based mobilization regimens and also the influence of post transplant rhG-CSF administration on peritransplant morbidity in patients undergoing auto-SCT.[11,14] However, sparse data exist about the use of late rhG-CSF after mobilization chemotherapy despite its potential for cost reduction and patient convenience. To our knowledge, to date, there is also no randomized study comparing different time schedules of rhG-CSF after mobilization chemotherapy. Therefore, in this randomized study, we attempted to compare two different timing schedules, early vs late, of the same dose of rhG-CSF in patients with hematological malignancies undergoing PBSC mobilization, to determine the influence of timing on stem cell yield.

Materials and methods

Patients

Between 2005 and 2008, 48 consecutive patients meeting protocol eligibility criteria with diagnoses of non-Hodgkin's lymphoma ($n = 22$) and multiple myeloma ($n = 26$) were entered on a trial evaluating different time schedules, early vs late, of rhG-CSF after chemotherapy for PBSC mobilization. Oral and written informed consent were obtained from all patients or their guardians. Patient characteristics are listed in Table 1. Patients were eligible for PBSC mobilization and harvest if they had an Eastern

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Cooperative Oncology Group performance status of 0–2, were aged $\leqslant 65$, and had evidence of adequate hepatic, renal and cardiac function.

Mobilization regimen

PBSC mobilization was performed with CE (CY 4 g/m^2 × 1 on day 1 and etoposide 200 mg/m^2/day × 1 on days 1, 2 and 3) in all patients. All patients received MESNA for the prevention of hemorrhagic cystitis. The MESNA dose was 4 g/m^2 given as equally divided doses before infusion of CY and 4 and 8 h afterwards. RhG-CSF 10 μg/kg/day i.v. twice daily was started at two time points after chemotherapy: on day 4 (early) and on day 7 (late) and continued until the last day of apheresis.[6,7,11] PBSC collection was started when the peripheral blood CD34$^+$ cell count reached 20/μl. CD34$^+$ cells/kg of 4×10^6/kg were considered an adequate harvest. Patients received antibiotic prophylaxis with ciprofloxacin 750 mg orally twice per day when the WBC count was $<0.5 \times 10^9$/l. Apheresis was performed using the Fenwall CS3000+ (Baxter Health Care System, Deerfield, IL, USA).

Table 1 Characteristics of the patients

Variable	*Day 4 (n = 25) Early group*	*Day 7 (n = 23) Late group*	P-*value*
Age	40.60 ± 12.20	48.00 ± 13.48	0.052
Sex			
Male	15 (60%)	15 (65.2%)	0.709
Female	10 (40%)	8 (34.8%)	
Diseases			
Multiple myeloma	11 (44%)	15 (65.2%)	0.141
Lymphoma	14 (56%)	8 (34.8%)	
Previous treatment			
Chemotherapy alone	21 (84%)	16 (69.6%)	0.235
Chemotherapy and RT	4 (16%)	7 (30.4%)	
Number of prior cycles of chemotherapy	6 (3–12)	6 (5–11)	0.673
Number of previously given different chemotherapy regimens (median, range)	2 (1–3)	2 (1–3)	0.407

Quantification of CD34$^+$ cells

Circulating peripheral blood CD34$^+$ cell counts before apheresis and CD34$^+$ cell counts collected in the apheresis product after each procedure were analyzed using flow cytometry. Erythrocytes were removed from samples of cells obtained before cryopreservation or after thawing and serial dilution by hypotonic lysis using ammonium chloride. Cells were then stained with a FITC or phycoerythrin-conjugated CD34 Ab (8G12; Becton Dickinson, San Jose, CA, USA) or an unrelated isotype control, washed and counterstained with propidium iodide (PI; Becton Dickinson) or 7-aminoactinomycin D (7AAD; Sigma Chemical, St Louis, MO, USA). After an additional wash and within 2 h of staining, the proportions of viable (PI or 7AAD excluding) cells, mononuclear cells and CD34$^+$ cells in the fresh and thawed specimens were determined by flow cytometric analysis (FACScan, Becton Dickinson). CD34$^+$ cells showed fluorescence of $>99.8\%$ of isotype control-stained cells. The quantities of these cell populations in each product before and after freezing were determined.[6,7,11]

Statistics

Data analyses were carried out using SPSS, version 15.00. χ^2 and Student's *t*-tests were used to compare proportions and means of continuous variables, respectively. Variables that did not fit a normal distribution were compared with the Mann–Whitney *U*-test. A *P*-value <0.05 (two-tailed) was considered to be statistically significant.

Results

Apheresis

No significant differences were observed between two groups in terms of sex, age and number of previously given different chemotherapy regimens. Although the late group of patients (day 7 group) was older and more frequently had myeloma, these differences did not reach statistical significance. There was a statistically significant difference between early and late groups regarding the time from chemotherapy to apheresis; 14 days (range 10–17) vs 15 days (range 10–21) ($P = 0.030$), respectively, (Table 2). The number of doses of rhG-CSF up to apheresis was

Table 2 Comparison of apheresis kinetics and median cell yields

	Day 4	*Day 7*	P-*value*
Time from chemotherapy to apheresis (median, range)	14 (10–17)	15 (10–21)	0.030
Number of doses of G-CSF (median, range)	20 (14–32)	16 (8–30)	0.005
Duration of neutropenia (median, range)	8 (4–14)	8 (5–11)	0.356
The day WBC count $\geqslant 1 \times 10^9$/l (mean ± s.d.)	12.56 ± 2.20	13.57 ± 2.96	0.187
WBC count on the first day of apheresis ($\times 10^9$/l)	8 (2.2–33.5)	7.6 (1.7–34.7)	0.820
CD34$^+$ count on the first day of apheresis per μl	75.84 (2–552)	37 (5–510)	0.263
TNC $\times 10^8$/kg	4.81 (1.77–12.72)	3.71 (1.30–15.91)	0.170
TMNC $\times 10^8$/kg	2.19 (1.19–4.87)	2.32 (0.70–11.65)	0.577
Total CD34$^+$ cells $\times 10^6$/kg	10.54 (0.11–37.27)	10.81 (0.17–49.83)	0.781
Number of apheresis	1 (1–3)	1 (1–3)	0.541
Patients with $\geqslant 2 \times 10^6$ CD34$^+$ cells/kg collected after one apheresis	19 (76%)	16 (72.7%)	0.797

Abbreviations: TMNC = total mononucleated cell; TNC = total nucleated cell.

significantly lower in the late group than in the early rh-GCF group. Median numbers of doses between the first dose of rhG-CSF and first cycle of apheresis in the early and late rhG-CSF groups were 20 doses (range 14–32) and 16 dozes (range 8–30), respectively ($P=0.005$) (Table 2). Duration of neutropenia between early and late groups, 8 days (range 4–14) vs 8 days (range 5–11), respectively, was not statistically significant ($P=0.356$). Table 2 gives the median days to WBC recovery of $>1\times10^9$/l after chemotherapy, WBC count on the day of leukapheresis and CD34$^+$ count on the first day of apheresis for both groups. No significant difference was observed between the two groups regarding duration of WBC recovery ($P=0.187$). WBC counts on the first day of apheresis in the early and late groups were 8×10^9/l (2.2–33.5) and 7.6×10^9/l (1.7–34.7), respectively, ($P=0.820$).

PBSC mobilization yields

The median CD34$^+$ cell count obtained on the first day of apheresis did not differ between the two arms: 75.84×10^9/l (2–552), 37×10^9/l (5–510), $P=0.263$. The number of leukapheresis procedures necessary to obtain optimal CD34$^+$ cell was also similar in both arms. PBSC mobilization yields are given in Table 2 and Figure 1 in terms of total CD34$^+$, total nucleated cell (TNC) and total mononucleated cell (TMNC) counts.

There were no statistically significant differences between the early and late rhG-CSF groups regarding the median numbers of daily means of TNCs, TMNCs and collected CD34$^+$ cell counts (Table 2 and Figure 1). Median numbers of daily means of TNCs, TMNCs and CD34$^+$ cells in the early and late rhG-CSF groups were 4.81×10^8/kg (range 1.77–12.72) vs 3.71×10^8/kg (range 1.30–15.91), 2.19×10^8/kg (range 1.19–4.87) vs 2.32×10^8/kg (range 0.70–11.65) and 10.54×10^6/kg (range 0.11–37.27) vs 10.81×10^6/kg (0.17–49.83), respectively, (Table 2).

In all, 76% of patients in the early rhG-CSF group and 73% in the late rhG-CSF group achieved $\geqslant2\times10^6$ CD34$^+$ cells/kg after one cycle of apheresis ($P=0.797$). The percentages of patients failing to achieve a collection of $\geqslant2.0\times10^6$/kg CD34$^+$ cells at the end of apheresis cycles in the early and late rhG-CSF groups were 16% ($n=4$) and 13% ($n=3$), respectively, and these were also not statistically significant ($P=1$).

In a subgroup analysis, although the numbers were small, there were also no differences among lymphoma patients in the early and late rhG-CSF groups regarding the collection yields. Similar results were also obtained for myeloma patients in the subgroup analysis.

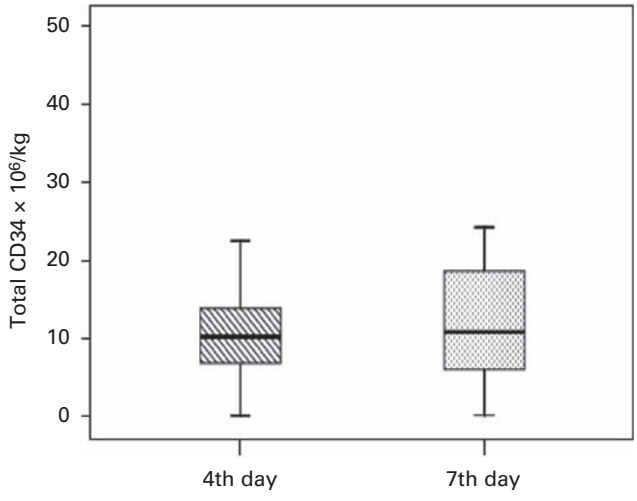


Figure 1 Comparison of total CD34$^+$ cell yields in the early and late recombinant human G-CSF groups.

Discussion

Despite many studies involving varied doses of rhG-CSF, the optimal timing for rhG-CSF administration after chemotherapy has not been conclusively defined to date and scant data are available regarding the late use of rhG-CSF despite the fact that this approach can reduce the cost attributed to cytokine use. Although in this paper, we used the term 'late' for day 7 administration of rhG-CSF, day 7 is frequently used for starting rhG-CSF in current practice.

Jacoub *et al.*[15] reported a pilot study describing the late use of rhG-CSF after chemotherapy in 28 patients with various malignancies. In that study, the 'late' use of rhG-CSF for PBSC mobilization was feasible and resulted in an adequate collection of CD34$^+$ cells suitable for auto-SCT at a potentially reduced cost. Jacoub *et al.*[15] also summarized studies involving 'late' rhG-CSF administration. It has to be noted that in most of these studies, the primary objective was not to evaluate 'late' administration of rhG-CSF.[16–21] These studies have evaluated the 'late' use of rhG-CSF (4 days after chemotherapy), at varied doses and schedules with comparable results with those obtained after starting rhG-CSF immediately after chemotherapy.[18,19,21]

Mollee *et al.*[16] compared CY + rhG-CSF with CE + rhG-CSF of 10 μg/kg once daily for PBSC mobilization in patients with lymphoma. In that study, the CY alone group received rhG-CSF on day 4 and CE + rhG-CSF group on day 6. The CE + rhG-CSF group was superior, however, in terms of total CD34$^+$cell yield. There was no difference between the two groups in terms of numbers of patients achieving a minimum threshold of $\geqslant2\times10^6$/kg CD34$^+$ cells.[16] Lefrere *et al.*[17] compared VAD + rhG-CSF of 10 μg/kg starting on day 10 with CY + rhG-CSF of 5 μg/kg starting on day 8 for PBSC mobilization in patients with multiple myeloma. In that study, the mobilization yields were equivalent between the two groups. The same proportion of patients achieved successful PBSC mobilization in both groups.[17] Min *et al.*[22] compared rhG-CSF starting on day 2 or 8 after infusion of CY in 30 patients, and they too found no difference between the two groups with regard to collection yield, toxicity or engraftment characteristics. Similarly, Haynes *et al.* and McQuaker *et al.*[18,19] reported the late administration of rhG-CSF after mobilization chemotherapy in patients with lymphomas. In both studies, patients achieved successful PBSC mobilization in terms of total CD34$^+$ cell yields.

In a different study, Lefrere *et al.*[23] reported 65 patients with multiple myeloma and non-Hodgkin's lymphoma who

underwent PBSC harvesting either with CY or CE + rhG-CSF. Initially, rhG-CSF was given immediately after chemotherapy on day 4. Later, it was administered on day 7 or 8. The median number of $CD34^+$ cells for one leukapheresis was not different between the two arms (2.4 vs 2.1, $P = 0.25$). The amounts of PBSCs harvested were also similar in the two groups.[23] However, the total rhG-CSF dose administered was one-third less with late introduction. Moreover, no increase in infection risk was observed with the late schedule. The authors concluded that the late introduction of rhG-CSF after chemotherapy might allow PBSC harvesting equivalent to that obtained after earlier rhG-CSF administration, without increasing the risk of infection.[23]

In our study, there were no statistically significant differences between the early and late rhG-CSF groups regarding median numbers of TNCs, TMNCs and collected $CD34^+$ cells. In all, 66% of patients in the early rhG-CSF group and 73% in the late rhG-CSF group achieved $\geqslant 2 \times 10^6$ $CD34^+$ cells/kg after one cycle of apheresis. Median numbers of doses between the first dose of rhG-CSF and first cycle of apheresis was significantly lower in the group of late rhG-CSF administration.

As far as we know, this is the first reported randomized study comparing the different time schedules of rhG-CSF after mobilization chemotherapy. In this study, the same mobilization regimen and the same dose of rhG-CSF were used in both patient groups. We may therefore speculate that this provided us with more consistent results compared with those studies reported previously using different doses, regimens and timing of rhG-CSF.[16–21,23–25]

We concluded that PBSC mobilization after the 'late' use of rhG-CSF is an effective approach, and that in routine clinical practice the late use of rhG-CSF may therefore be used for PBSC collections after chemotherapy-based mobilization regimens in this cost-conscious era.

Conflict of interest

The authors declare no conflict of interest.

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