Bone Marrow Transplantation (2000) 25, 931–935
© 2000 Macmillan Publishers Ltd All rights reserved 0268–3369/00 $15.00
www.nature.com/bmt

Single *vs* twice daily G-CSF dose for peripheral blood stem cells harvest in normal donors and children with non-malignant diseases

V Lee[1], CK Li[1], MMK Shing[1], KW Chik[1], K Li[1], KS Tsang[2], DC Zhao[1], DH Lai[1], A Wong[1] and PMP Yuen[1]

Departments of [1]Paediatrics and [2]Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China

Summary:

The optimal dose and schedule of G-CSF for mobilization of peripheral blood stem cells (PBSC) is not well defined. G-CSF mobilization was performed in a group of healthy donors and paediatric patients for autologous back-up before receiving allogeneic stem cell transplant. Seventeen consecutive subjects who received G-CSF at 5 μg/kg/dose twice daily (group A) were compared with a historical control group of 25 subjects who received a single daily dose of 10 μg/kg/day G-CSF (group B). Double blood volume apheresis for PBSC collection was started on day 5. G-CSF was continued and apheresis repeated until the targeted CD34$^+$ cell dose was achieved. Both groups were comparable for sex, age, body weight and reason for PBSC collection. Over two-thirds of the subjects in both groups were less than 16 years of age. The G-CSF priming and apheresis were well tolerated. When the first day apheresis products were analyzed, group A resulted in significantly higher yield of total nucleated cells (5.91 *vs* 3.92 × 10^8/kg, $P = 0.013$), mononuclear cells (5.73 *vs* 3.92 × 10^8/kg, $P = 0.017$), CD34$^+$ cells (2.80 *vs* 1.69 × 10^6/kg, $P = 0.049$) and colony-forming units (107 *vs* 54 × 10^4/kg, $P = 0.010$) as compared with group B. We conclude that the two dose schedule is more efficient in mobilizing PBSC in normal donors and children with non-malignant diseases. This approach may reduce the number of aphereses required and thus reduce the transplant cost. *Bone Marrow Transplantation* (2000) **25,** 931–935.

Keywords: G-CSF; PBSC harvest; healthy donors; children

Mobilized peripheral blood stem cells (PBSC) can durably restore normal haematopoietic function when transplanted after myeloablative therapy.[1–3] With more rapid engraftment, less serious infections and less blood product support, autologous PBSC transplant has now replaced autologous bone marrow transplant in most centres. Recently, PBSC has also been used for allogeneic transplantation,[4,5] avoiding the requirement for general anaesthesia for the donors and allowing rapid haematopoietic reconstitution in the patients. PBSC may also be harvested and stored as autologous backup in patients who have a high chance of graft rejection after allogeneic stem cell transplant, such as thalassaemia major and non-malignant diseases for unrelated donor transplant. Autologous backup PBSC can be re-infused into patients to reconstitute haematopoietic function if necessary. Mobilization of PBSC from healthy donors and non-cancer patients who have not been exposed to radio-chemotherapy is largely achieved by recombinant human granulocyte colony-stimulating factor (G-CSF).[6,7] However, the optimal dose and schedule of G-CSF priming for PBSC mobilization in normal donors and non-cancer patients has not been determined. Most of the early studies administered G-CSF as single daily dose, but there were a few studies using divided doses every 12 h.[8] We report here our experience of using G-CSF in two divided doses a day as compared with a single daily dose in mobilization of stem cells.

Materials and methods

Donors and patients

Seventeen consecutive normal PBSC donors and non-cancer patients were recruited into the study from January 1998 to July 1999. PBSC from normal donors were collected for HLA-identical or one antigen mismatched related donor transplantation. PBSC from non-cancer patients were collected for autologous backup before proceeding to allogeneic stem cell transplantation if they were considered to have a high chance of graft rejection, such as thalassaemia major and unrelated donor transplant for metabolic diseases. Informed written consent for administration of G-CSF and collection of PBSC was obtained from the donors, patients or the parents before the procedure. These 17 subjects were given 10 μg/kg/day recombinant human non-glycosylated G-CSF (Filgastrim, Amgen, Thousand Oaks, CA, USA) subcutaneously in two divided doses a day (2 × 5 μg/kg, group A). The result was compared with a historical control group of 23 subjects (group B) who had PBSC collection during the period January 1996 to December 1997. In group B, the subjects received subcutaneous G-CSF 10 μg/kg as single daily doses. Complete blood counts were monitored daily during G-CSF treat-

---

Correspondence: Dr CK Li, Department of Paediatrics, Lady Pao Children's Cancer Centre, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China

Received 16 November 1999; accepted 16 January 2000

ment, and the dose of G-CSF was reduced by 50% if the total white cell count rose above 70 × 10^9/l.

PBSC collection

The subjects in both groups received G-CSF for 4 days. The first apheresis was performed in the morning of day 5. The methodology of PBSC harvest was identical in both groups. Stem cells were harvested with Fenwal CS3000 (Baxter Healthcare, Deerfield, IL, USA) continuous flow cell separator. The blood volume processed for each apheresis was at least twice the total blood volume. The minimum number of $CD34^+$ cell required was 3×10^6/kg recipient body weight for allogeneic stem cell transplant from healthy donors, and 1×10^6/kg patient body weight for autologous backup. Administration of G-CSF was continued and apheresis repeated every morning until the targeted number of $CD34^+$ cells had been obtained.

Evaluation of PBSC products

Blood counts and differentials from peripheral blood and apheresis products were performed with a Coulter automated cell counter (STKS; Coulter Corp., Miami, FL, USA). $CD34^+$ stem and progenitor cells were measured by a FACScan flow cytometer (Becton Dickinson (BD), San Jose, CA, USA). Apheresis products containing 1×10^6 cells were lysed with the FACS lysing solution (BD) according to manufacturer's protocol. These cells were stained with CD34-fluorescein isothiocyanate (FITC) and CD38-phycoerythrin (PE) antibodies. A total of 78 000 cells was acquired for enumerating $CD34^+$ cells using Lysis II software (BD). The mononuclear cell (MNC) population was identified in another sample stained with CD45-FITC and CD14-PE. The number of $CD34^+$ cells was expressed as the percentage of the cells in the MNC gate after subtracting the background events stained with isotypic control antibodies. All antibodies were products of BD. Colony-forming units were enumerated by cell culture. PBSC (2×10^5, 1×10^5 and 5×10^4) were seeded in 35-mm culture dishes of 1.35% methylcellulose (1500 centipoises) enriched with 30% fetal calf serum, 1% bovine serum albumin, 100 μM β-mercaptoethanol, and a recombinant human cytokine cocktail of 1 IU erythropoietin Eprex, 20 ng stem cell factor and 100 ng recombinant granulocyte–macrophage colony-stimulating factor (GM-CSF). Triplicate cultures were incubated in 5% CO_2 incubator at 37°C for 2 weeks. Colonies of over 40 cells were scored.

Statistics

Results were expressed as median and range per kg body weight of the subjects. Since the required number of aphereses depended on the purpose of PBSC and the body weight of recipients, we only compared the yield of PBSC products in the first apheresis according to the subjects' body weight between the two groups. Comparison between groups was done with standard statistical tests, proportions were compared using chi-squared tests and non-parametric data were compared using the Mann–Whitney rank sum test. The relationship between age and body weight with $CD34^+$ cell count was estimated by Spearman correlation analysis. A two-tailed P value <0.05 was considered to be statistically significant.

Results

Donor and patient characteristics

The characteristics of the healthy donors and non-cancer patients are shown in Table 1. All the healthy donors were HLA-identical or one antigen mismatched siblings or parents. The underlying diseases of the non-cancer patients were thalassaemia major ($n = 21$), mucopolysaccharidosis ($n = 2$) and adrenoleukodystrophy ($n = 2$). The youngest patient was a 1-year-old boy who weighed 8 kg at the time of PBSC collection. Twelve and 15 subjects in groups A and B were younger that 16 years old, respectively. Group A and group B were comparable for sex, age, body weight and the purpose of PBSC harvest.

G-CSF administration

Administration of G-CSF was given according to protocol in all subjects. No abortion of G-CSF priming was required. The side-effects of G-CSF priming were mild and limited to fever, myalgia and bone pain which were readily relieved by paracetamol.

PBSC collection

A total of 52 aphereses was performed. In group A, two required two aphereses and two required three. In group B, six required two aphereses and none required three. The median blood volume processed per apheresis was 5.5 l

Table 1 Basic characteristics of normal donors and non-cancer patients according to schedule of G-CSF

	Group A *2 × 5 μg/kg/day*	*Group B* *1 × 10 μg/kg/day*	*P value*
Number	17	23	
Sex			
Male	9	8	
Female	8	15	0.409
Age (year)			
<16 years	12	15	
⩾16 years	5	8	0.986
Range	1–53	5–48	
Median	8	13	0.286
Body weight (kg)			
Range	8–83	15–74	
Median	23.3	34.9	0.325
Purpose			
Allotransplant[a]	9	6	
Auto backup[b]	8	17	0.160

[a] Healthy donors for allogeneic PBSC transplant were either HLA-identical or one antigen mismatched related donors.

[b] Non-cancer patients for autologous PBSC backup before receiving allogeneic stem cell transplantation. Two had mucopolysaccharidosis, two adrenoleukodystrophies, and the remaining had transfusion-dependent thalassaemia.

(range, 2 to 16.6 l) and was comparable in both groups (group A *vs* group B, 5.5 *vs* 6.0). This did not differ when only the first aphereses were analysed (group A *vs* group B, 5.4 *vs* 6.0, $P = 0.721$). The target CD34$^+$ cell dose could be achieved in all the subjects after one to three aphereses.

None of the subjects required a second PBSC mobilization or bone marrow harvest subsequently to obtain more stem cells. Numbness, nausea and muscle cramps developed in three of the subjects during apheresis, respectively. The symptoms were readily relieved by slow intravenous injection of 10% calcium gluconate. No other clinically significant electrolyte disturbance was observed during and after each apheresis. Peripheral blood platelet count dropped in most of the subjects after apheresis, but clinically significant thrombocytopenia was not observed. The peripheral blood cell counts returned to normal within a few days in all the subjects.

Analysis of the first apheresis

Table 2 summarizes the yield from the first apheresis collection from both groups. The total nucleated cells (TNC), MNC, CD34$^+$ cells and CFU were significantly higher in group A than group B. The median cell numbers and CFU of group A were higher than group B: TNC 5.91 *vs* 3.92 $\times 10^8$/kg; MNC 5.73 *vs* 3.92 $\times 10^8$/kg; CD34$^+$ cells 2.80 *vs* 1.69 $\times 10^6$/kg; CFU, 107 *vs* 54 $\times 10^4$/kg. While the percentage of MNC in TNC (%MNC/TNC) and the percentage of CD34$^+$ cells in MNC (%CD34$^+$/MNC) were not statistically different between the two groups. The median %MNC/TNC and %CD34$^+$/MNC of group A and B were 95.0 *vs* 98.0% and 0.71 *vs* 0.47%, respectively. Since the response to G-CSF might be different between allo-transplant and auto-backup patients, the analysis was also done on group A and group B separately. There was no difference in the yield of progenitor cells (TNC, MNC, CD34$^+$ cells and CFU) between the allo-transplant and auto-backup subsets for both group A and group B.

Table 2 Comparison of first apheresis products after mobilization with 2 × 5 μg/kg (group A) with 1 × 10 μg/kg (group B)

	Group A *2 × 5 μg/kg/day*	*Group B* *1 × 10 μg/kg/day*	*P value*
TNC ($\times 10^8$)/kg)			
Median	5.91	3.92	0.013
Range	2.61–10.19	1.18–6.87	
%MNC in TNC			
Median	97	98	0.547
Range	82–100	82–100	
MNC ($\times 10^8$/kg)			
Median	5.73	3.92	0.017
Range	2.29–10.19	0.17–1.73	
%CD34$^+$ cells/MNC			
Median	0.710	0.470	0.357
Range	0.27–1.69	0.17–1.73	
CD34$^+$ cells ($\times 10^6$/kg)			
Median	2.80	1.69	0.049
Range	0.68–11.39	0.51–7.53	
CFU ($\times 10^4$/kg)			
Median	107	54	0.010
Range	13–344	18–173	

TNC = total nucleated cells; MNC = mononuclear cells; CFU = colony-forming units.
Values were expressed as per kg body weight of the donor/patient.

The influences of sex, age and body weight of the subjects and the purpose of PBSC collection on the yield of CD34$^+$ cells in the first aphereses were analysed. There was no correlation between the number of CD34$^+$ cell harvested and the age, gender or body weight of the subjects. The median numbers of CD34$^+$ cell collected in the first aphereses for allo-transplant group and auto-backup groups were 2.05 $\times 10^6$/kg (range, 0.68–10.17 $\times 10^6$/kg) and 2.20 $\times 10^6$/kg (range, 0.51–11.39 $\times 10^6$/kg) respectively. There was no significant difference between the two groups ($P = 0.84$).

Discussion

Despite being widely used in PBSC transplant, the optimal dose and schedule of G-CSF for mobilization of PBSC in normal donors or non-oncology patients has not been established yet. Previous studies have demonstrated that efficacy of PBSC mobilization by G-CSF is dose-dependent.[6,9–11] Doses of 3–5 μg/kg/day result in less efficient PBSC mobilization,[9,12] and high doses at 16–24 μg/kg/day may not be cost-effective and may be associated with increased toxicity. The EBMT has recommended that normal donors undergoing mobilization for allogeneic PBSC transplant should receive G-CSF 10 μg/kg as single daily doses for 5 days with PBSC collection starting 1 day after the fourth dose.[13] Mobilization kinetic studies have shown that the number of stem cells starts to rise between 48 to 72 h after initiation of G-CSF treatment and reaches a maximum level between days 4 and 6 of treatment.[6,14] These results support the use of single daily dose schedule of G-CSF with apheresis performed on the fifth day of G-CSF treatment. However, the pharmacological profile of G-CSF shows the maximum serum concentration after subcutaneous injection is reached within 2–8 h and the elimination half-life is only 3 to 4 h.[15] Hence application of G-CSF once daily may be suboptimal for PBSC mobilization. The MD Anderson Cancer Center has shown in more than 350 donors that G-CSF 12 μg/kg/day given in two divided doses a day is effective in mobilizing PBSC.[8] There have been no studies comparing the single against divided dose schedule of G-CSF on the yield of PBSC collection in normal donors and non-cancer patients. Here we evaluated the efficacy of G-CSF in PBSC mobilization with a two divided dose a day schedule and compared with a historical once daily dose schedule. Our results suggest that, at the same daily dose of G-CSF of 10 μg/kg/day, a two divided dose a day schedule allows a significantly greater number of stem cells and colony-forming units to be collected than the single daily dose schedule.

In a retrospective study on 49 heavily pretreated breast cancer patients, a two 5 μg/kg doses daily schedule of G-CSF results in a higher yield of CD34$^+$ cells and fewer aphereses than the 10 μg/kg once daily schedule.[16] Another prospective study on 48 consecutive normal PBSC donors has shown that G-CSF at doses of 6–8 μg/kg/12 h provides better PBSC mobilization and collection than

10 μg/kg/day,[17] but the difference in actual daily doses of G-CSF may have confounded the results. In the current study, we used the same daily dose of GCSF of 10 μg/kg/day. We analysed both the healthy donors for PBSC transplant and non-cancer patients for autologous PBSC backup together. Impaired mobilization of PBSC has been reported in some patients with inherited, non-malignant, haematopoietic disorders such as chronic granulomatous disease and adenosine deaminase deficient severe combined immunodeficiency disease.[18] The results suggest that the mobilization kinetics may be different between healthy donors and patients who suffered from non-malignant diseases. The majority of our non-cancer patients suffered from transfusion-dependent thalassaemia and none suffered from immunodeficiency syndromes. We have previously shown that the yield of $CD34^+$ cells from thalassaemia patients does not differ from normal healthy donors.[19] Furthermore, in the current study the yield of $CD34^+$ cells from the healthy donors did not differ from the non-cancer patients. The results suggest that the mobilization kinetics of the two groups are similar.

The result of this study must be interpreted with caution because it was compared with a historical control group. Even though a uniform methodology was adopted throughout the years, there may be other confounding factors causing a difference in the yield of stem cells. A randomised control trial is the preferred method to confirm the efficacy of the twice daily dose schedule. Our subjects were relatively young (two-thirds being less than 16 years), this may explain the insignificance of an age effect as reported by others.[20–22] We did not find any differences in the incidence and severity of side-effects between subjects receiving twice a day or once a day doses. Fourteen allogeneic PBSC transplants have been performed and the rate of engraftment was not affected by the dose schedule of G-CSF for mobilisation.

In conclusion, giving G-CSF as two divided doses a day appeared to achieve higher yields of $CD34^+$ cells and CFU than a single daily dose. This approach may lead to a lower number of aphereses required to obtain sufficient $CD34^+$ cells. A two divided doses a day schedule may be more cost-effective for PBSC mobilization.

References

1 To LB, Roberts MM, Haylock DN *et al*. Comparison of haematological recovery times and supportive care requirements of autologous recovery phase peripheral blood stem cell transplants, autologous bone marrow transplants and allogeneic bone marrow transplants. *Bone Marrow Transplant* 1992; **9**: 277–284.

2 Schwartzberg L, Birch R, Blanco R *et al*. Rapid and sustained hematopoietic reconstitution by peripheral blood stem cell infusion alone following high-dose chemotherapy. *Bone Marrow Transplant* 1993; **11**: 369–374.

3 Schmitz N, Linch DC, Dreger P *et al*. Randomised trial of filgrastim-mobilized peripheral blood progenitor cell transplantation versus autologous bone marrow transplantation in lymphoma patients (see comments) (published erratum appears in *Lancet* 1996; **347**: 914). *Lancet* 1996; **347**: 353–357.

4 Bensinger WI, Clift R, Martin P *et al*. Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: a retrospective comparison with marrow transplantation. *Blood* 1996; **88**: 2794–2800.

5 Schmitz N, Bacigalupo A, Hasenclever D *et al*. Allogeneic bone marrow transplantation *vs* filgrastim-mobilized peripheral blood progenitor cell transplantation in patients with early leukaemia: first results of a randomised multicentre trial of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 1998; **21**: 995–1003.

6 Dreger P, Haferlach T, Eckstein V *et al*. G-CSF-mobilized peripheral blood progenitor cells for allogeneic transplantation: safety, kinetics of mobilization, and composition of the graft. *Br J Haematol* 1994; **87**: 609–613.

7 Russell JA, Luider J, Weaver M *et al*. Collection of progenitor cells for allogeneic transplantation from peripheral blood of normal donors. *Bone Marrow Transplant* 1995; **15**: 111–115.

8 Anderlini P, Donato M, Chan KW *et al*. Allogeneic blood progenitor cell collection in normal donors after mobilization with filgrastim: the MD Anderson Cancer Center experience. *Transfusion* 1999; **39**: 555–560.

9 Grigg AP, Roberts AW, Raunow H *et al*. Optimizing dose and scheduling of filgrastim (granulocyte colony-stimulating factor) for mobilization and collection of peripheral blood progenitor cells in normal volunteers (see comments). *Blood* 1995; **86**: 4437–4445.

10 Hoglund M, Smedmyr B, Simonsson B *et al*. Dose-dependent mobilization of haematopoietic progenitor cells in healthy volunteers receiving glycosylated rHuG-CSF. *Bone Marrow Transplant* 1996; **18**: 19–27.

11 Waller CF, Bertz H, Wenger MK *et al*. Mobilization of peripheral blood progenitor cells for allogeneic transplantation: efficacy and toxicity of a high-dose rhG-CSF regimen. *Bone Marrow Transplant* 1996; **18**: 279–283.

12 Dreger P, Haferlach T, Eckstein V *et al*. G-CSF-mobilized peripheral blood progenitor cells for allogeneic transplantation: safety, kinetics of mobilization, and composition of the graft. *Br J Haematol* 1994; **87**: 609–613.

13 Russell NH. The place of blood stem cells in allogeneic transplantation. *Br J Haematol* 1996; **93**: 747–753.

14 Stroncek DF, Clay ME, Herr G *et al*. The kinetics of G-CSF mobilization of $CD34^+$ cells in healthy people. *Transfus Med* 1997; **7**: 19–24.

15 de Haas M, Kerst JM, van der Schoot CE *et al*. Granulocyte colony-stimulating factor administration to healthy volunteers: analysis of the immediate activating effects on circulating neutrophils. *Blood* 1994; **84**: 3885–3894.

16 Kroger N, Zeller W, Hassan HT *et al*. Stem cell mobilization with G-CSF alone in breast cancer patients: higher progenitor cell yield by delivering divided doses (2 × 5 μg/kg) compared to a single dose (1 × 10 μg/kg). *Bone Marrow Transplant* 1999; **23**: 125–129.

17 Arbona C, Prosper F, Benet I *et al*. Comparison between once a day *vs* twice a day G-CSF for mobilization of peripheral blood progenitor cells (PBPC) in normal donors for allogeneic PBPC transplantation. *Bone Marrow Transplant* 1998; **22**: 39–45.

18 Sekhsaria S, Fleisher TA, Vowells S *et al*. Granulocyte colony-stimulating factor recruitment of $CD34^+$ progenitors to peripheral blood: impaired mobilization in chronic granulomatous disease and adenosine deaminase-deficient severe combined immunodeficiency disease patients. *Blood* 1996; **88**: 1104–1112.

19 Li K, Wong A, Li CK *et al*. Granulocyte colony-stimulating factor-mobilized peripheral blood stem cells in beta-thalassemia patients: kinetics of mobilization and composition of apheresis product. *Exp Hematol* 1999; **27**: 526–532.

20 Anderlini P, Przepiorka D, Seong C *et al*. Factors affecting mobilization of $CD34^+$ cells in normal donors treated with filgrastim. *Transfusion* 1997; **37**: 507–512.

21 Miflin G, Charley C, Stainer C *et al*. Stem cell mobilization in normal donors for allogeneic transplantation: analysis of safety and factors affecting efficacy. *Br J Haematol* 1996; **95**: 345–348.

22 Demirer T, Buckner CD, Bensinger WI. Optimization of peripheral blood stem cell mobilization. *Stem Cells* 1996; **14**: 106–116.