Peripheral blood stem cells used to augment autologous bone marrow transplantation

P L R Mitchell, V B Shepherd, H M Proctor, M Dainton, S D Cabral, C R Pinkerton

Abstract

Peripheral blood stem cells (PBSC) were used to augment autologous bone marrow transplantation (ABMT), aiming to hasten engraftment after high dose treatment in a group of heavily pretreated patients. PBSC were obtained by leukapheresis during the rebound after standard chemotherapy. In 11 patients aged 7-17 years, high dose chemotherapy consisted of busulphan 16 mg/kg orally with melphalan 160 mg/m² intravenously for seven patients, and melphalan 200 mg/m² intravenously alone for four. The median number of granulocyte-macrophage colony forming units in the reinfused PBSC was $3.42 \times 10^4/\text{kg}$ (3.03-18.01) and bone marrow $12.4 \times 10^4/\text{kg}$ (4.16-28.6). Neutrophil recovery to $\geq 0.5 \times 10^9/\text{l}$ and platelet transfusion independence occurred at a median of 14 days (11-18) and 22 days (9-84) respectively. In five patients the early engraftment was transient with neutrophils again dropping below $0.5 \times 10^9/\text{l}$ then slowly recovering. There was one toxic death due to sepsis. PBSC harvesting in these children was undertaken without interrupting routine chemotherapy and without the use of bone marrow growth factors. In some patients PBSC failed to influence engraftment and the use of combined chemotherapy and growth factor priming for PBSC collection may give improved results.

(*Arch Dis Child* 1994; 70: 237-240)

Dose escalation has become an important strategy in the treatment of childhood cancers and in a number of diseases attention has focused on high dose chemotherapy with autologous bone marrow rescue.¹ Stem cells derived from the peripheral circulation may also be used to achieve haematopoietic reconstitution.^{2,3}

There are several potential advantages of transplantation using peripheral blood stem cells (PBSC).⁴ Patients with hypocellular bone marrow due to prolonged chemotherapy or pelvic radiotherapy may be unsuitable for marrow harvesting. Sufficient progenitor cells can be obtained by PBSC collection even though the marrow is hypocellular and the harvesting of clinically undetectable malignant cells in marrow may be less likely. However, the major advantage of PBSC transplantation is rapid haematopoietic reconstitution.⁵ In

addition to stem cells, committed progenitor cells capable of early maturation are collected by leukapheresis. These committed progenitor cells are a possible source of endogenous growth factors evident before PBSC engraftment.⁶

After myelosuppressive chemotherapy there is a massive increase in circulating progenitor cell levels.⁷ The accurate timing of PBSC collections with this surge improves progenitor cell yields.⁸⁻¹⁰ An alternative method of priming for PBSC collection involves use of the marrow growth factors granulocyte colony stimulating factor (G-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF).¹¹

A major factor in the toxicity of high dose treatment with autologous bone marrow transplantation (ABMT) is the period of myelosuppression, particularly in heavily pretreated patients. In order to achieve sustained engraftment after PBSC transplantation a number of PBSC harvest procedures may be required. A study in adults suggested that by combining PBSC transplantation and ABMT the number of leukaphereses could be reduced and a benefit from rapid, if transient, engraftment, was demonstrated.¹² Eventual permanent engraftment was achieved later by the ABMT, but the combination reduced early morbidity.

With the aim of reducing the duration of neutropenia and thrombocytopenia, PBSC were similarly used in the current study to augment bone marrow rescue in a group of heavily pretreated patients. PBSC collections were timed to coincide with the surge of progenitor cells after routine chemotherapy. As the PBSC transplant was not intended to replace ABMT only two collections were planned without using marrow growth factors in order to minimise disruption of the regular chemotherapy schedule.

Patients and methods

PATIENTS AND HIGH DOSE CHEMOTHERAPY REGIMENS

Eleven patients aged 7-17 years received combined PBSC transplantation and ABMT (table 1). There were five patients with Ewing's sarcoma, three with rhabdomyosarcoma, and one patient each with T cell lymphoma, acute myeloid leukaemia, and angiosarcoma of the liver. High dose treatment was used as consolidation of chemotherapy induced remission except for one patient with T cell lymphoma who was treated in relapse.

**Royal Marsden
Hospital, Paediatric
Unit**
P L R Mitchell
C R Pinkerton

Leukaemia Unit
V B Shepherd
H M Proctor
M Dainton
S D Cabral

Correspondence and
requests for reprints to:
Dr Ross Pinkerton,
Department of Paediatrics,
The Royal Marsden
Hospital, Downs Road,
Sutton, Surrey SM2 5PT.

Accepted 8 November 1993

Table 1 Characteristics of 11 patients undergoing combined PBSC and ABMT

Case No	Age/sex	Diagnosis	Primary (metastases)	Status*	Preparation	Outcome†
1	12/F	Acute myeloid leukaemia		CR	Busulphan/melphalan	FOD 24 months
2	7/F	T cell lymphoma	Mediastinum (bone marrow)	PD	Busulphan/melphalan	DOD 22 days
3	17/F	Ewing's sarcoma	Foot (lung, lymph nodes)	PR	Busulphan/melphalan	Toxic death 51 days
4	10/M	Ewing's sarcoma	Pelvis (lung)	CR	Busulphan/melphalan	DOD 8 months
5	12/M	Ewing's sarcoma	Ileum	CR	Busulphan/melphalan	FOD 36 months
6	16/M	Rhabdomyosarcoma	Pelvis (bone marrow, lymph nodes)	PR	Melphalan	DOD 6 months
7	9/F	Ewing's sarcoma	Femur (bone)	CR	Busulphan/melphalan	FOD 17 months
8	14/M	Ewing's sarcoma	Pelvis (lung)	CR	Busulphan/melphalan	FOD 18 months
9	14/M	Rhabdomyosarcoma	Arm (bone marrow, lymph nodes)	CR	Melphalan	AWD 9 months
10	16/F	Angiosarcoma	Liver (liver)	PR	Melphalan	FOD 6 months
11	15/M	Rhabdomyosarcoma	Cheek	Second CR	Melphalan	FOD 6 months

*CR=complete remission, PD=progressive disease, PR=partial remission.

†AWD=alive with disease, DOD=died of disease, FOD=free of disease.

Patients received busulphan 16 mg/kg orally over four days followed by melphalan 160 mg/m² intravenously, or melphalan 200 mg/m² given alone (table 1).

and rapidly reinfused by syringe. Patients received intravenous hydration during reinfusion. The day of reinfusion was designated as day 0.

SUPPORTIVE CARE

Patients were isolated in filtered air rooms, although parents had free access. No particular procedures were followed regarding food and no gut sterilisation was used. Antifungal prophylaxis comprised oral amphotericin and nystatin, or fluconazole. All patients had a Hickman line with at least two lumens.

Pneumocystis prophylaxis was commenced at neutrophil recovery, with co-trimoxazole then being continued for six months.

Patients were transfused to maintain haemoglobin >90 g/l and platelets >20×10⁹/l. Cytomegalovirus IgG negative patients received only cytomegalovirus negative blood products, and all blood products were irradiated.

BONE MARROW AND PBSC COLLECTION

Bone marrow was harvested and cryopreserved except for two patients receiving high dose melphalan alone who were transplanted with fresh marrow. The marrow was processed to give a buffy coat concentrate and Ficoll separation was undertaken in some of the younger children to reduce volume and red cell contamination.

Leukapheresis was carried out following routine myelosuppressive chemotherapy and was timed to coincide with a total white cell count of 2×10⁹/l. It has been previously shown that after such chemotherapy a total white cell count of 2×10⁹/l with rapidly increasing monocyte numbers identifies the peak in peripheral blood CFU-GM levels.¹⁰

Two leukapheresis sessions were planned. The stem cell programme of the COBE Spectra cell separator was used to process 1.5–2 times the estimated blood volume, producing about 1 ml/min of stem cell product.

PBSC and bone marrow for cryopreservation underwent controlled freezing with 5% dimethyl sulphoxide, and were stored in the vapour phase of liquid nitrogen. The cryopreserved products were thawed at the bedside

CFU-GM CULTURE

Cells were cultured in soft agar at 5×10⁴ nucleated cells per plate to determine CFU-GM content; 10% 5637 conditioned medium was used as growth factor. After incubation at 37°C for 14 days in a carbon dioxide incubator, the plates were scored for CFU-GM colonies of at least 50 cells. CFU-GM data were available only in those where marrow was cryopreserved.

Results

PBSC harvesting was successfully carried out in all 11 patients with no complications, however, two patients required more than two collections. Most patients had PBSC harvested at between 16 and 19 days after chemotherapy.

Table 2 outlines the levels of PBSC and bone marrow progenitor cell levels reinfused, and the times to neutrophil and platelet recovery. Patients undergoing ABMT received PBSC with a median CFU-GM count of 3.42×10⁴/kg (range 3.03–18.01). The median CFU-GM count of bone marrow reinfused was 12.4×10⁴/kg (range 4.16–28.6).

Reconstitution of haematopoiesis occurred in all patients. Neutrophils reached 0.5×10⁹/l at a median of 14 days (range 11–18 days), and platelet transfusion independence was achieved at a median of 22 days (range 9–84 days). No correlation was observed between days to neutrophil or platelet recovery and infused levels of PBSC or bone marrow nucleated or CFU-GM cells (linear regression analysis for neutrophil recovery, log rank test for time to platelet transfusion independence).

Neutrophil recovery was well sustained in four patients (cases 2, 6, 9, 11), while for two patients there was a minor dip in neutrophil levels (cases 1, 4). However, the other five patients showed only transient early engraftment, with neutrophils dropping again at day 16–19 below 0.5×10⁹/l (figure).

One patient (case 5) with neutrophil recovery to 0.6×10⁹/l at day 14, dropped to

Table 2 CFU-GM levels of transplanted cells and haematological recovery

Case No	Duration of chemotherapy before harvest (months)		PBSC harvests	CFU-GM $\times 10^4/\text{kg}$		Days to recovery		
	PBSC	Bone marrow		PBSC	Bone marrow	Neutrophils $\geq 0.5 \times 10^9/\text{l}$	Platelet transfusion independent	Days in hospital
1	5	5	2	6.66	28.6*	12	18	31
2	7	7	2	4.66	12.88*	14	22+	22 (died)
3	3	4	2	18.01	18.9	14	36	51 (died)
4	3	4	2	9.79	7.6*	13	9	22
5	7	6	2	3.42*	13.6*	14	50	36
6	4	7	2	3.31	10.53	16	25	23
7	6	3	2	3.03	12.4	17	48	23
8	1	3	3	3.38	4.16	11	11	19
9	3, 4	7	4	5.30	—	16	20	20
10	4	6	2	3.35	—	18	84†	58
11	3‡	0‡	2	3.34	9.4	12	15	14

*Mononuclear cell preparation using Ficoll separation.

†Platelets remained $< 20 \times 10^9/\text{l}$ at 6 months.

‡Second chemotherapy course for relapse.

0.1 at day 21 and remained with neutrophils $< 0.5 \times 10^9/\text{l}$ for a further 26 days. Another patient (case 10) was given G-CSF $5 \mu\text{g}/\text{kg}/\text{day}$ IV (Amgen Roche, Cambridge) before engraftment from days 10–17 in view of marked oral mucositis and uncontrolled sepsis. Neutrophil recovery to $1.0 \times 10^9/\text{l}$ occurred by day 18 but neutrophils then dropped to 0.3 at day 21 on stopping G-CSF. G-CSF was later reintroduced from days 27–34 but she remained in hospital for 58 days. The neutrophil level of the final patient (case 3) dropped from $1.6 \times 10^9/\text{l}$ at day 16 to 0.4 on day 23, but recovered to $> 1.0 \times 10^9/\text{l}$ with G-CSF $5 \mu\text{g}/\text{kg}/\text{day}$ given intravenously from days 19–24. However, she continued to be unwell with marked oral mucositis and subsequently deteriorated with bacterial pneumonitis. She died of pulmonary haemorrhage during artificial ventilation.

All 11 patients required broad spectrum antibiotic treatment for sepsis while neutropenic, and all but one had World Health Organisation grade 3–4 oral mucositis.

Follow up is short, but three patients with Ewing's sarcoma remained alive and free of disease at 17–36 months (table 1). A further patient with Ewing's sarcoma was in complete

remission at necropsy after her toxic death at day 51 as outlined above. One patient with acute myeloid leukaemia was free of disease at 24 months, one patient with hepatic angiosarcoma was free of disease at six months, and one patient with relapsed rhabdomyosarcoma remained in a second remission for six months.

Discussion

This patient group was selected because it was anticipated that ABMT reconstitution would be slow in view of their intensive prior chemotherapy. Even transient neutrophil recovery at an early stage would be advantageous in reducing septic complications at a time when other treatment related toxicity such as mucositis and enteral toxicity are prominent. This strategy appeared to be successful in most of our patients.

Leukapheresis was successfully carried out in these 11 paediatric patients without disruption of their usual chemotherapy routine. For most patients only two leukapheresis sessions were required to collect $> 3.0 \times 10^4/\text{kg}$ CFU-GM.

Neutrophil recovery to $\geq 0.5 \times 10^9/\text{l}$ was prompt at a median of 14 days. This compares favourably with an earlier group of similar patients receiving ABMT alone in whom the median time was 18 days (range 14–27) (unpublished data).

Stable platelet engraftment occurred at a median of 22 days in the current group. As expected, using bone marrow in addition to PBSC, no late graft failure was seen, but one patient remained with platelets $< 20 \times 10^9/\text{l}$ at six months. However, in seven patients the early neutrophil recovery was not sustained, and dropped below $0.5 \times 10^9/\text{l}$ again in five patients due to only transient engraftment of the PBSC reinfusion.

For some of these children the addition of PBSC to ABMT apparently failed to reduce the toxicity of the procedure, and in particular platelet engraftment was hastened in only a minority.

For successful transplantation using autologous PBSC alone it has been suggested that $10\text{--}30 \times 10^4/\text{kg}$ CFU-GM is required in paediatric patients.³ However, there is considerable individual variation and as little as $0.7 \times 10^4/\text{kg}$ CFU-GM may be sufficient in

Patients with neutrophil counts subsequently falling below $0.5 \times 10^9/\text{l}$. Patients receiving G-CSF were case 3 (days 19–24) and case 10 (days 10–17, days 27–34).

some cases.³ In the current study there was no clear relationship between the stability of the early neutrophil rise and the infused PBSC CFU-GM count, although four out of five patients with unstable early engraftment received $3-3.5 \times 10^4/\text{kg}$ CFU-GM. None of these patients had a history of bone marrow involvement by tumour, which is known to be associated with slow engraftment. Likewise there was no clear association between the duration of chemotherapy before leukapheresis and the stability of PBSC engraftment.

Early platelet engraftment has been the major advantage of PBSC reinfusion in adults. However, in several studies patients were less heavily pretreated than these children, a factor likely to influence platelet engraftment in particular. PBSC collections were carried out during the rebound after chemotherapy without the use of growth factors. The use of colony stimulating factors before collection has now been shown to improve progenitor cell yields,¹¹ and our current practice is to utilise PBSC obtained by combined chemotherapy and G-CSF priming for four days before and during harvest. The use of marrow growth factors add significantly to the cost of the procedure and this study demonstrates that even without their use there is potential benefit.

The degree of cytoreduction achievable with a single course of high dose treatment is limited. Although in haematopoietic malignancies this may be curative this is less likely in solid tumours. In adults PBSC reinfusion allows repeated sequential high dose treatment,¹³ a strategy that is feasible in the paediatric setting and could improve the poor prognosis of some paediatric tumours.

The precise advantages of PBSC reinfusion over ABMT in children have yet to be clarified. In the young child there may be little to choose between a general anaesthetic for marrow harvest and several hours on a cell separator. Randomised studies evaluating engraftment timing and procedure costs are needed. In patients with active marrow disease there is

likely to be little advantage to PBSC rescue compared with ABMT. High dose treatment will be ineffective in either case and failure is due to chemoresistant disease in the child, not contamination of the reinfused marrow.

- 1 Ladenstein R, Hartmann O, Pinkerton CR. The role of megatherapy with autologous bone marrow rescue in solid tumours of childhood. *Ann Oncol* 1993; 4 (suppl 1): S45-S58.
- 2 Fakuda M, Kojima S, Matsumoto K, et al. Autotransplantation of peripheral blood stem cells mobilized by chemotherapy and recombinant human granulocyte colony-stimulating factor in childhood neuroblastoma and non-Hodgkin's lymphoma. *Br J Haematol* 1992; 80: 327-31.
- 3 Takaue Y. Peripheral blood stem cell autografts in children with acute lymphoblastic leukaemia and lymphoma: updated experience. *Leuk Lymphoma* 1991; 3: 241-56.
- 4 Lowry PA, Tabbara IA. Peripheral hematopoietic stem cell transplantation: current concepts. *Exp Hematol* 1992; 20: 937-42.
- 5 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: 277-84.
- 6 Kawano Y, Takaue Y, Saito SI, et al. Granulocyte colony-stimulating factor (CSF), macrophage-CSF, granulocyte-macrophage CSF, interleukin-3, and interleukin-6 level in sera from children undergoing blood stem cell autografts. *Blood* 1993; 81: 856-60.
- 7 Richman CM, Weiner RS, Yankee RA. Increase in circulating stem cells following chemotherapy in man. *Blood* 1976; 6: 1031-9.
- 8 Craig JIO, Smith SM, Parker AC, et al. The response of peripheral blood stem cells to standard chemotherapy for lymphoma. *Leuk Lymphoma* 1992; 6: 363-8.
- 9 Emminger W, Emminger-Schmidmeier W, Hocker P, et al. The median daily increment of leukocytes during haematopoietic recovery reflects the myeloid progenitor cell yield during leukapheresis in children. *Bone Marrow Transplant* 1990; 5: 419-24.
- 10 Fernandez JM, Shepherd V, Millar J, et al. When is the optimum time to harvest peripheral blood stem cells in children following standard dose chemotherapy? *Med Pediatr Oncol* 1993; 21: 465-9.
- 11 Haas R, Hohaus S, Egerer G, et al. Recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) subsequent to chemotherapy improves collection of blood stem cells for autografting in patients not eligible for bone marrow harvest. *Bone Marrow Transplant* 1992; 9: 459-65.
- 12 Gianni AM, Bregni M, Siena S, et al. Rapid and complete haematopoietic reconstitution following combined transplantation of autologous blood and bone marrow cells. A changing role for high dose chemo-radiotherapy? *Hematol Oncol* 1989; 7: 139-48.
- 13 Shea TC, Mason JR, Storniolo AM, et al. Sequential cycles of high-dose carboplatin administered with recombinant human granulocyte-macrophage colony-stimulating factor and repeated infusions of autologous peripheral-blood progenitor cells: a novel and effective method for delivering multiple courses of dose-intensive therapy. *J Clin Oncol* 1992; 10: 464-73.