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Pre infusion, post thaw CD34$^+$ peripheral blood stem cell enumeration as a predictor of haematopoietic engraftment in autologous haematopoietic cell transplantation

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ABSTRACT

Introduction: By convention, peripheral blood stem cell products for autologous transplantation are evaluated for quality by CD34$^+$ cell dose at the time of harvesting. A CD34$^+$ cell dose in excess of 2.0×10^6/kg of recipient body weight is considered adequate for haematopoietic engraftment. Viable CD34$^+$ cell numbers are enumerated in most laboratories using the ISHAGE single platform flow cytometric method which utilizes monoclonal antibodies to CD45, CD34 and 7 amino actinomycin D (7AAD) dye exclusion.

Methods: One hundred and six consecutive autologous transplantation procedures underwent viable CD34$^+$ cell enumeration at the time of harvesting and post thaw prior to reinfusion. Neutrophil and platelet engraftment and markers of haematopoietic support were analyzed.

Results: Mean pre-cryopreservation viable CD34$^+$ numbers were 4.882×10^6/kg. Mean post thaw viable CD34$^+$ numbers were 3.234×10^6/kg. Mean loss of viable CD34$^+$ cells with processing and cryo-preservation was 1.648×10^6/kg (33%). For neutrophil engraftment, there was no significant difference between high ($\geqslant 3.0 \times 10^6$/kg) and low ($<1.5 \times 10^6$/kg) post thaw viable CD34$^+$ cell counts ($p = 0.545$). For platelet engraftment, there was however a significant difference observed between the high and low pre infusion viable CD34$^+$ groups ($p < 0.001$). Additionally, significant differences were seen between the post thaw viable CD34$^+$ cell count and the associated length of hospital admission, days of use of G-CSF post transplantation, use of antibiotics in the post transplantation period and transfusion support in the post transplantation period.

Conclusion: A significant loss of viable CD34$^+$ cells occurs during processing, cryopreservation and thawing. Low numbers of viable CD34$^+$ cells infused post thaw will still result in adequate neutrophil engraftment however may delay platelet engraftment. Low viable CD34$^+$ cell numbers have significant effects on admission duration and use of haematopoietic supportive measures with consequent effects on healthcare resources.

1. Introduction

Peripheral Blood Haematopoietic Stem Cells (PBSC) are now the commonest source of CD34$^+$ stem cells used for transplantation after myeloablative therapy for a variety

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of malignant and non-malignant conditions [1]. The success of this technique is dependent upon the ability of the PBSC product to reconstitute haematopoiesis following mobilization, leukapheresis, laboratory processing, cryopreservation, thawing and reinfusion [2].

The CD34 antigen phenotypically defines a diverse population of stem and progenitor cells capable of short and long-term multi-lineage haematopoietic reconstitution in humans following myeloablative cytotoxic therapy [3]. One of the key determinants of adequate engraftment and haematopoietic reconstitution is the $CD34^+$ cell dose. A threshold quantity of 2.0×10^6 $CD34^+$ cells per kilogram of recipient body weight at the time of collection has been established as a reliable predictor of successful neutrophil and platelet engraftment, and is now incorporated into transplantation guidelines for use when assessing the quality of PBSC products [4]. There is however uncertainty regarding the effect of cryopreservation and processing on the quality of PBSC products and whether an appreciable loss of $CD34^+$ numbers has a clinically significant effect on haematopoietic reconstitution and engraftment [5,6].

The International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol is the most widely adopted single platform method of $CD34^+$ cell enumeration in which cell number and viability are evaluated. ISHAGE methodology is now routinely employed at the time of graft collection to determine the quality of the PBSC product [7].

The aims of this study were to prospectively investigate the magnitude of loss of viable $CD34^+$ cell numbers with processing, cryopreservation and thawing, and whether this has a significant effect on the time to haematopoietic engraftment and other surrogate markers of haematopoietic reconstitution.

Table 1
Patient characteristics.

Age (yrs; mean and range)	49 (17–67)
Sex (M/F)	53%/47%
Diagnosis (*n*)	
Myeloma	47
NHL	40
Hodgkin's lymphoma	8
Amyloidosis	1
Germ cell tumours	3[a]
Multiple sclerosis	6
Ewing's sarcoma	1
Mean inpatient length of stay (d)	15.98
Mean red cell concentrate (units/patient)	3.03
Mean platelet concentrate (units/patient)	2.20
Mean days of GCSF use	10.3
Mean days of IV antibiotics	5.52
Preparative regimens	
BEAM	Carmustine 300 mg/m^2 D-6, etoposide 200 mg/m^2 D-5–D-2, Cytrabine 200 mg/m^2 D-5–D-2, melphalan 140 mg/m^2 D-1
HD melphalan	Melphalan 200 mg/m^2 D-1
BEAM–ATG	Carmustine 300 mg/m^2 D-6, etoposide 200 mg/m^2 D-5–D-2, Cytrabine 200 mg/m^2 D-5–D-2, melphalan 140 mg/m^2 D-1, ATG (rabbit) 3.75 mg/kg D + 1–D + 2
Carboplatin–etoposide	Carboplatin 700 mg/m^2 D-5–D-3
	etoposide 750 mg/m^2 D-5–D-3
Busulfan–melphalan	Busulfan 1.6 mg/kg D-7–D-6
	Busulfan 3.2 mg/kg D-5–D-3
	Melphalan 140 mg/m^2 D-2

[a] Patients with germ cell tumours underwent dual tandem autografting with the carboplatin/etoposide regimen.

2. Materials and methods

2.1. Patients, PBSC mobilization, collection and transplantation protocols

From a single transplant centre, 140 autologous PBSC products from 106 consecutive patients were obtained between June 2008 and September 2012. Indications for autologous stem cell transplantation included haematologic malignancy, solid malignancy and multiple sclerosis (Table 1).

PBSC were mobilized with a variety of protocols dependent on patient specific factors and physician preference. These included:

1. 2 g/m^2 of cyclophosphamide with 10 μg/kg Granulocyte-Colony Stimulating Factor (G-CSF) for 11+/−2 days
2. 4 g/m^2 of cyclophosphamide with 10 μg/kg G-CSF for 11+/−2 days
3. Ifosfamide, carboplatin and etoposide and 10 μg G-CSF for 11+/−2 days
4. Any of the above with plerixafor for 0.24 mg/kg for 1–5 days.

Mobilization efficacy was monitored by assessment of peripheral blood $CD34^+$ numbers using flow cytometry (Becton–Dickinson FacsCalibur™ cell analyzer, San Jose, California, USA) and leukapheresis was undertaken when peripheral blood $CD34^+$ counts were greater than 10/μL in peripheral blood.

Leukapheresis was undertaken using either a COBE® Spectra Apheresis system, or from 2009, a COBE® Spectra Optia apheresis system (Terumo BCT, California, USA) with up to 12–20 L of blood being processed for each collection. Target viable $CD34^+$ PBSC yield was $\geqslant 2.0 \times 10^6$/kg of recipient body weight.

Preparative regimens for transplantation varied with the diagnosis. Patients with Non-Hodgkin and Hodgkin lymphoma received bis-chloroethylnitrosourea (BCNU), etoposide, cytarabine and melphalan (BEAM). Those with myeloma and amyloidosis received high dose melphalan and those with germ cell tumours received carboplatin and etoposide. Those with refractory multiple sclerosis received modified BEAM with rabbit anti thymocyte globulin. A single patient with refractory Ewing's Sarcoma underwent pre autograft conditioning with busulfan and melphalan (Table 1).

PBSC products were re-infused 24–48 h after completion of the preparative regimen and G-CSF routinely administered at 5 μg/kg from day +1 following re-infusion

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