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ARTICLE

Freezing the graft is not necessary for autotransplants for plasma cell myeloma and lymphomas

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Abstract

We studied rates of granulocyte and platelets recovery in 359 consecutive subjects receiving blood cell infusions in the context of autotransplants for plasma cell myeloma ($N = 216$) and lymphomas ($N = 143$). Blood cells were mobilised with filgrastim given for 4–5 days and collected after a median of 2 (range, 1–2) apheresis. Apheresis products were stored at 4° C for a median of 3 days (range, 2–6 days). Most subjects received carmustine, etoposide, cytarabine and melphalan (BEAM), cyclophosphamide, carmustine and etoposide (CBV) or high-dose melphalan. Filgrastim was given post transplant to 319 subjects. Median numbers of mononuclear cells collected was $31 \times 10E + 6$/kg (interquartile range (IQR) $37 \times 10E + 6$ cells/kg). Median numbers of CD34-positive cells collected was $3.6 \times 10E + 6$/kg (IQR $3.8 \times 10E + 6$/Kg). Median viability after collection was 90% (IQR 7%) after storage, 88% (IQR 12%). A total of 255 of 256 evaluable subjects recovered bone marrow function and there was no late bone marrow failure. Median interval to neutrophils $>0.5 \times E + 9$/L was 13 days (range, 9–39 days) and to platelets $>20 \times 10E + 9$/L, 16 days (range, 7–83 days). These rates and ranges seem comparable to those reported after autotransplants of frozen blood cells. There was no correlation between numbers of storage days at 4 °C and viability afte storage ($r = -0.018$, $p = 0.14$)) nor rates of recovery of neutrophils ($r = -0.054$, $p = 0.52$) or platelets ($r = 0.116$, $p = 0.14$). Blood cells collected for autotransplant can be stored at 4 °C for 6 d. This method is simple, inexpensive and widely applicable.

Introduction

High-dose therapy followed by autotransplant is widely used to treat plasma cell myeloma and lymphomas [1, 2]. Conventional procedures for freezing blood cells for an autotransplant are complex, time-consuming and expensive [3]. We and others reported blood cells collected for an autotransplant survive up to 7 days at 4 °C in a conventional blood blank refrigerator [4–9]. We now report a multicentre, international experience using this method.[10, 11]

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Subjects and methods

Subjects

Data of consecutive subjects with plasma cell myeloma or lymphoma receiving an autotransplant January 1, 2002 to December 31, 2016 in transplant centres in Argentina ($N = 2$), Colombia ($N = 1$) and México ($N = 2$) were analysed. Written informed consent was obtained from all subjects compliant with policies of each centre.

Definitions and evaluation

Neutrophil recovery was defined as the 1st of ≥3 consecutive days with neutrophils >0.5 × 10E+/L. Platelet recovery was defined as the 1st day of platelets ≥20 × 10E+/L for three consecutive days without platelet transfusions.

Aphereses

Aphereses used an Amicus® (Fresenius Kabi, Deerfield, IL, USA) or a Spectra Optia® devices (Terumo BCT, Lakewood, CO, USA) and the Spin–Nebraska protocol [6]. The apheresis goal was to collect ≥2 × 10E + 6/kg viable CD34-postive cells. Aphereses products and 1 mL aliquots were stored in ACD-A® (Baxter Healthcare, Deerfield, IL USA) at 4 °C, in 1 L transfer packs (Baxter Healthcare) composed of gas impermeable, polyvinyl chloride plastic film, or in the same collection bag of the apheresis kit (Terumo BCT Spectra Optia Apheresis System) for up to 6 d. Enumeration of mononuclear cells (MNC) and CD34-positive cells was done by flow-cytometry in an EPICS® device (Coulter Electronics, Hialeah, FL USA), using phyco-erythrin labelled anti-CD34 HPCA-2 monoclonal antibody (Becton Dickinson, San Jose, CA USA) and a fluorescence iso-thiocyanate tagged anti-CD45 monoclonal antibody (Beckman Coulter, Hialeah, FL USA) gating in 7′amino-actinomycin. Viability of stored MNC was tested by propidium iodide or tryptan blue exclusion in a flow cytometer or under light microscopy. Aphereses products were stored in in a conventional blood bank refrigerator (Thermoforma, Marietta, OH, USA).

Transplant protocols

Plasma cell myeloma

Blood cell mobilisation used granulocyte colony-stimulating factor (G-CSF; filgrastim),10 µg/kg/d for 4–5 consecutive days [2]. Sixteen subjects also received cyclophosphamide, 1.5 g/mE + 2/d for 2 days and 11, plerixafor, 0.24 mg/kg, 11 h pre-aphereses. One or two consecutive apheresis each processing 3–5 blood volumes were done to obtain ≥2 × 10E + 9/kg CD34-positive cells. Collections were stored at 4 °C in a blood bank refrigerator for up to 6 d. Subjects received melphalan, 200 ($N = 211$) or 140 mg/mE + 2 ($N = 5$) over 1 d. A total of 29 subjects also received cyclophosphamide, 2.4 g/mE + 2, for 1–2 consecutive d. Blood cells were infused 24 h after the last drug dose. A total of 176 subjects received G-CSF, 5 µg/kg/d, starting 3–5 days post-infusion and continued until neutrophils were >0.5 × 10E+/L.

Lymphoma

Filgrastim, 10 or 15 µg/kg/d, for 4 or 5 days was used to mobilise blood cells. Apheresis, processing and storage were as above [12]. Details of the conditioning regimens are reported [1, 7]. Blood cells were infused on day 0. Filgrastim was given to all lymphoma subjects as above.

Statistical analyses

Descriptive statistics were used for subject-related, disease-related and transplant-related variables using SPSS version 2.0 software, including IQR calculations. Data were tested for normality using the Kolmogorov–Smirnov test [13]. This was followed by the Spearman correlation coefficient to assess correlations between numbers of days of storage, CD34-positive cell recovery, viability and rate of recovery of neutrophils and platelets [14]. $P < 0.05$ was considered significant.

Results

Subjects

A total of 123 subjects with plasma cell myeloma were male with a median age of 54 years (range, 29–75 years). Eighty six subjects with lymphoma were male with a median age of 39 years (range, 5–67 years). Lymphoma diagnoses included diffuse large B-cell lymphoma ($N = 64$; 45%), Hodgkin lymphoma ($N = 63$; 44%) and mantle cell lymphoma ($N = 16$; 11%). Lymphoma subjects were in ≥2nd remission except 16 with mantle cell lymphoma in 1st remission. Median follow-up of survivors is 427 days (range, 60 days–14 years).

Cell recovery

Median numbers of MNC collected was 31.0 × 10E + 8/kg (IQR 36.8 × 10E + 8/kg). Median numbers of CD34-postive cells collected was 3.6 × 10E + 6/kg (IQR 3.8 × 10E + 6/kg). Cells were stored at 4 °C for a median of

Table 1 Comparison of rates of neutrophil and platelet recoveries

	Reference	Storage	*N*	Neutrophils >0.5 × 10E + 9/L	Platelets >20 × 10E + 9/L
Plasma cell myeloma	18	Frozen	201	–	16 (132–19)
	19	Frozen	221	11 (2–25)	18 (1–95)
	This study	4° C	216	14 (9–39)	16 (7–83)
Lymphoma	20	Frozen	20	14 (10–18)	14 (10–22)
	19	Frozen	217	10 (8–30)	19 (9–384)
	21	Frozen	146	11 (10–12)	15 (12–26)
	This study	4° C	143	12 (9–10)	17 (9–38)

3 days (range, 2–6 days) in subjects with myeloma and a median of 6 days (range, 5–6 days) in subjects with lymphoma. Median cell viability after collection was 90% (IQR, 7%) and after storage, 88% (IQR 12%). There was no correlation between storage interval and cell viability after storage ($r = -0.018$; $p = 0.84$).

Recovery of bone marrow function

Median interval to neutrophils $>0.5 \times 10E + 9/L$ was 14 days (IQR 4 days) in subjects with plasma cell myeloma and 12 days (IQR 2 days) in subjects with lymphomas ($P > 0.5$). Median interval to platelets $>20 \times 10E + 9/L$ was 16 days (IQR 7 days) and 17 days (IQR 10 d; $P = 0.04$), respectively. There was bone marrow recovery in 216 subjects with plasma cell myeloma (three subjects died, one at day +7, and 2 at day +9) and in 140 subjects with lymphomas (one subject did not recover by day +60 and received an allotransplant). There was no significant correlation between days of storage at 4 °C and interval to recovery of neutrophils ($r = -0.054$, $p = 0.52$) or platelets ($r = 0.116$; $p = 0.14$).

Discussion

Considerable data support the efficacy of blood cell grafts stored at 4 °C for several days [4–9, 15, 16]. This approach is simpler and less expensive than freezing cells and eliminates exposure of the recipient to cryo-protective drugs such as dimethyl-sulfoxide. The Table shows data of bone marrow recovery in persons with plasma cell myeloma and lymphoma receiving autotransplants of frozen or cells stored at 4 °C [17–21]. Recovery times for neutrophils and platelets are similar. We found no correlation between numbers of days of 4 °C storage and CD34-positive cell recovery, viability or rates of neutrophil and platelet recovery. However, the *P*-values of 0.14 for rate of platelet recovery suggest a statistical model other than the null hypothesis might better explain these data (Table 1).

Neutrophil and platelet recovery times for subjects with plasma cell myeloma and lymphomas were similar despite plasma cell myeloma subjects receiving fewer bone marrow damaging drugs before aphereses. Bone marrow recovery after an autotransplant derives from the infused cells and recovery of endogenous, surviving bone marrow cells. Because bone marrow suppression from high-dose melphalan is far greater than from CBV or BEAM, similar rates may reflect different balances in these pathways of bone marrow recovery.

Analyses of outcomes of autotransplants in our subjects other than bone marrow recovery such as progression-free survival or survival was not the object of our study. Having shown rapid, sustained bone marrow recovery using grafts stored at 4 °C these other outcomes depend on subject-related, transplant-related and disease-related variables, not conditions of graft storage. Analyses of these outcomes in persons receiving grafts stored at 4 °C or frozen requires a large, randomised, double-blind clinical trials.

There are several potential limitations to our study. First, it was retrospective. Second, we did not quantify numbers of mononuclear cells nor CD34-positive cells post-storage. However, the rapid, sustained recovery of bone marrow function indicates persistence of adequate numbers of both. Lastly, we did not have a randomised, double-blind control cohort against which to compare other autotransplant outcomes which could be affected by storage method but seems unlikely.

We see three potential limitations to storing at 4 °C: (1) if the autotransplant had to be postponed, say after the 1st or 2nd dose of chemotherapy. However, this is rare and did not occur in any of the 359 consecutive subjects in our study. In such an instance the refrigerated haematopoietic cells could then be frozen or additional aphereses done; (2) if the blood cell collection was scheduled for a long interval before the planned autotransplant. This is sometimes done in which case the technique we describe would not be appropriate; (3) if the aphereses were intended to collect cells for more than one autotransplant. In this instance storage at 4 °C might be appropriate for the 1st transplant whereas cells for later transplants could be frozen.

In conclusion, storing haematopoietic cells at 4 °C could expand autotransplants to centres where more complex technical skills and equipment are lacking and/or where cost may be an issue. It could also reduce complexity and cost at centres presently freezing cells for an autotransplant. It is unclear why this method is not more commonly used.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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