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The relationship between CD34+ stem cell dose and time to neutrophil recovery in autologous haematopoietic stem cell recipients—A single centre experience

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ABSTRACT

A retrospective, observational study was performed of 112 patients who underwent autologous haematopoietic stem cell transplantation (ASCT) to determine the relationship between CD34+ stem cell dose and neutrophil engraftment. Importantly, a novel approach to more accurately calculate time to neutrophil engraftment was employed. The results demonstrated that a higher CD34+ stem cell dose was associated with faster neutrophil recovery ($P < 0.05$). CD34+ stem cell dose using actual and ideal patient body weight were both equally predictive of neutrophil engraftment as were absolute and viable CD34+ measurements. The clinical implications for this relationship are limited with an increase in CD34+ stem cell dose by 1×10^6/kg reducing the neutrophil engraftment time by only 3 h and 50 min. The median time to neutrophil recovery was 217 h (9 days and 1 h) and this relatively early engraftment time may be related to an early initiation of granulocyte colony-stimulating factor (G-CSF) on day +1 post-transplant. Female patients engrafted 17 h faster than their male counterparts on multi-variate analysis ($P < 0.05$). Conditioning chemotherapy, bacteraemia, G-CSF dose/kg body weight and increasing age had no impact on time to neutrophil recovery.

1. Introduction

Autologous stem cell transplantation (ASCT) is integral to the treatment of various malignant haematological conditions. Multiple myeloma remains the most common indication for high-dose chemotherapy with autologous stem cell support, followed by non-Hodgkin lymphoma [1]. It is important for clinicians to recognise factors associated with time to haematopoietic recovery after myeloablative chemotherapy. This in turn can lead to optimising supportive care during the cytopenic period.

Peripheral blood stem cells (PBSC) are typically enumerated using post-collection CD34 antigen positive stem cells [2]. Though there is no optimal CD34+ cell dose that is recommended in ASCT, current practice mandates a minimum dose of 2 million CD34+ cells/kilogram (kg) body weight [3]. It has been previously demonstrated that infusions of higher CD34+ cells/kg body weight are associated with faster haematopoietic recovery after myeloablative chemotherapy [4–6]. Specifically, retrospective studies have shown that high CD34+ cells/kg body weight are associated with faster platelet engraftment but to date there are conflicting results on the association with neutrophil engraftment [7,8]. This may in part be related to a difficulty in establishing the precise time that neutrophil engraftment was attained.

In addition to the dose of peripheral blood stem cells infused, other treatment-related factors may influence the time to haematopoietic recovery. These include the conditioning chemotherapy regimen and use of cytokine support in the form of granulocyte colony-stimulating factor (G-CSF) [9,10]. There continues to be differing views in the published literature on the role and potential benefits of G-CSF support post ASCT [9]. Viable CD34+ enumeration at time of re-infusion has also been postulated to correlate better with engraftment [7]. However, there is no consensus in the current literature on whether viable CD34+ cell count is a better predictor of myeloid recovery after autotransplantation than absolute CD34+ cell count [11].

In the context of these observations, we sought to determine the relationship between CD34+ cells/kg body weight and time to neutrophil engraftment in hours. We also assessed for the most predictive CD34+ cell count (absolute vs. viable) in determining neutrophil engraftment. Further, secondary factors (treatment and patient-related

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factors) that may have impacted on neutrophil recovery were evaluated.

2. Materials and methods

2.1. Study design

This was a single centre, retrospective, observational study performed at the Townsville Hospital, Queensland, Australia to determine whether there was a relationship between CD34+ cell dose and time to neutrophil recovery. The study has been approved by the Human Research Ethics Committee, Townsville Hospital and Health Service.

2.2. Study participants

Between August 2012 and August 2017, 131 adult patients underwent ASCT. 112 patients were included in the analysis and individual patient characteristics, conditioning regimens and episodes of bacteraemia during the cytopenic period were collected. 19 patients who were excluded from the study comprised those with solid-organ malignancy as a transplantation indication, those requiring split dose PBSC infusions and patients who died prior to engraftment. All patients received G-CSF from day +1 post-transplant until neutrophil engraftment. As per our institutional policy, G-CSF was administered at 300 mcg for patients < 80 kg or 480 mcg if ≥80 kg.

2.3. CD34+ cell count

Absolute CD34+ and viable CD34+ cell enumeration harvest was performed using a validated no-lyse/no-wash method [12], BD consumables (TruCount tubes, CD45 FITC, CD34 PE and 7-AAD), and the International Society of Haematology and Graft Engineering (ISHAGE) single-platform gating strategy [13] on a FACS CANTO II flow cytometer (BD Biosciences San Jose, California, USA). The absolute CD34+ cell count was derived from an aliquot taken from the fresh apheresis collection. The viable CD34+ cell count was derived from a thawed aliquot of the cryopreserved collection.

The CD34+ cell count per kg body weight was determined using the patient's ideal and actual body weight to allow for comparative analysis. The patients' ideal body weight (IBW) was calculated using the Devine formula [14]. Each patient had 4 measures of CD34+ cell counts: (1) absolute CD34+ cell count/kg actual body weight (ABW), (2) absolute CD34+ cell count/kg ideal body weight (IBW), (3) viable CD34+ cell count/kg ABW and (4) viable CD34+ cell count/kg IBW. The minimum required CD34+ cells/kg actual body weight at our institution is 2.5×10^6/kg.

2.4. Determining neutrophil recovery

Time to neutrophil recovery was defined as the duration of time to first achieve a neutrophil count $\geq 0.5 \times 10^9$/L after PBSC infusion. Importantly, the unit of time was measured in hours, and not days. The neutrophil count needed to be maintained at $\geq 0.5 \times 10^9$/L for 72 h to meet the definition of neutrophil engraftment. Platelet engraftment was not assessed in this study.

Complete blood count profiles were performed on a daily basis. The time of each blood collection was determined in hours post commencement of stem-cell infusion. Daily absolute neutrophil counts were then graphically represented for each patient (x-axis: hours post PBSC infusion, y-axis: neutrophil count) and a linear trend-line equation was generated. The trend-line equation was then used to accurately calculate the precise time to neutrophil recovery (extrapolated neutrophil engraftment time). A case example of calculating extrapolated engraftment time is provided in the Supplementary material.

2.5. Statistical methods

Continuous variables were tested for normality and based on the outcome, non-parametric analyses with Man Whitney U and Spearman's rho for correlation analysis was undertaken. Factors affecting engraftment time were assessed using multivariate linear regression. A P-value of < 0.05 was considered statistically significant. All analyses were performed using Statistical Package for the Social Sciences (SPSS®), Version 24.

3. Results

3.1. Patients

Of the 112 patients analysed, the median age was 59 years (range, 20–71). 57% of patients were men, 66% had multiple myeloma, 28% non-Hodgkin lymphoma, and 6% Hodgkin lymphoma. Conditioning chemotherapy was determined by disease entity with 60% of patients receiving melphalan and 32% receiving BEAM (BCNU, etoposide, cytarabine, melphalan) +/− rituximab. The remaining 8% received busulphan/melphalan or carmustine/thiotepa for multiple myeloma and primary CNS lymphoma respectively. Of the patients who received melphalan, 96% received melphalan at a dose of 200 mg/m^2 and 4% at a dose of 140 mg/m^2. Patient characteristics are shown in Table 1.

3.2. CD34+ cell count and neutrophil engraftment

The median time to extrapolated neutrophil engraftment was 217 h (9 days and 1 h). The median absolute CD34+ cell count was 4.16×10^6/kg ABW and 5.29×10^6/kg IBW. The median viable CD34+ cell count was 3.14×10^6/kg ABW and 3.98×10^6/kg IBW. A significant relationship was maintained between all four CD34 cell count measurements and time to extrapolated neutrophil engraftment ($P < 0.01$). This was deemed to be a moderate correlation for all four CD34+ measurements [15] with Spearman's $\rho = 0.39$ for absolute CD34+ cell count/kg ABW, $\rho = 0.44$ for absolute CD34+ cell count/kg IBW, $\rho = 0.34$ viable CD34+ cell count/kg ABW and $\rho = 0.39$ for viable CD34+ cell count/kg IBW ($P < 0.001$) – Table 2. On univariate analysis, for every increase in CD34+ cell count by 1×10^6/kg there was a reduction in neutrophil engraftment by 4 h and 12 min ($P < 0.01$) – Fig. 2.

3.3. Secondary factors and neutrophil engraftment

There was no significant difference in time to neutrophil recovery amongst the four conditioning regimens. Median time to extrapolated neutrophil engraftment was 214 h (range, 209–231) with melphalan, 218 h (range, 211–234) with BEAM +/− rituximab, 216 h (range, 185–249) with busulphan/melphalan, and 191 h (range, 189–191) with carmustine/thiotepa (Fig. 1).

Multivariate linear regression analysis showed no significant impact

Table 1
Baseline Characteristics of patients included in the analysis.

Age, median (range)	59 (20–71)
Male sex, no. (%)	64 (57%)
Indication for Transplant, n (%)	
Multiple Myeloma	74 (66%)
Non-Hodgkin lymphoma	31 (28%)
Hodgkin lymphoma	7 (6%)
Conditioning Regimen, n (%)	
Melphalan	67 (60%)
BEAM +/− R	36 (32%)
Other	9 (8%)
Presence of Bacteraemia, n (%)	21 (19%)