

Graft Composition and Post-Thawing Cell Viability Influence the Hematopoietic Recovery in Autologous Hematopoietic Stem Cell Transplantation

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Abstract

Objective: We investigated the influence of graft composition and post-thawing cell viability on the hematopoietic recovery of patients undergoing Autologous Hematopoietic Stem Cell Transplantation (ASCT).

Methods: Data relative to 146 ASCT procedures performed in 134 patients were examined. The doses of CD34+ cells and parameters related to the grafts' composition (white blood cell – WBC, neutrophil and platelet -PLT concentrations) were correlated to the number of days for neutrophil and platelet engraftment. Moreover, patients were grouped according to the values of post-thawing total nucleated cell (TNC) and CD34+ cell viability (as lower or higher than median values of the entire series) and hematopoietic recovery was accordingly evaluated.

Results: The CD34+ cell dose significantly predicts both neutrophil and PLT engraftment. Patients with low TNC viability had longer time to neutrophil engraftment, while patients with low CD34+ cell viability exhibited longer time to both neutrophil and PLT engraftment. High WBC or neutrophil concentrations in the graft were associated with low TNC viability. In addition, we found an inverse correlation between CD34+ cell viability and graft PLT concentration.

Conclusions: Our findings suggest that the platelet content in apheresis products is a critical issue, with an impact on CD34+ cell viability and hematopoietic recovery after ASCT. The apheresis products containing high amounts of PLT should be evaluated for PLT removal before freezing.

Keywords: CD 34+ cells; Total nucleated cells; Viability; Hematopoietic stem cell transplantation; Engraftment

Introduction

Hematopoietic Stem Cell Transplantation (HSCT) is a potentially curative treatment for several hematological diseases. An adequate number of total nucleated and CD34+ cells in the graft plays a pivotal role to achieve sustained engraftment and good survival. However, recent evidences in the allogeneic HSCT setting suggest that additional factors, from cellular graft composition to freezing and storage procedure, determine the graft quality and, ultimately, influence the outcome of patients [1,2]. In the field of Autologous Stem Cell Transplantation (ASCT), the attention has mainly focused on the amount of transplanted cells: it is widely acknowledged that a clear relationship exists between dose of CD34+ cells infused and engraftment [3-6]. On the contrary, the effects of graft characteristics on clinical outcome have been sparsely investigated [7-9]. Although previous study showed that the loss of CD34+ during cryopreservation has clinically significant effects on hematopoietic reconstitution [10], there is uncertainty regarding which factors are potentially detrimental for cell viability during cryopreservation.

In this study we evaluated the influence of graft composition and post-thawing cell viability on the hematopoietic recovery of patients undergoing ASCT. Moreover, we attempted to clarify which graft characteristics are associated with reduced cell viability.

Patients and Methods

Patients

Between January 2011 and December 2014, 134 patients (median age 54 years, range 19-66; male/female:77/57) undergoing ASCT were enrolled in the study. Underlying diseases were Non Hodgkin

lymphoma (NHL) in 59 patients, multiple myeloma (MM) in 55, and Hodgkin lymphoma (HL) in 20. Twelve patients with MM underwent two sequential ASCTs. Peripheral blood stem cells (PBSCs) were harvested by leukapheresis (COBE Spectra, TerumoBCT, Lakewood, CO, USA), after mobilization with high dose chemotherapy and G-CSF. In all patients a single apheresis product per transplant was utilized. The engraftment times for neutrophils (i.e. the time to achieve an absolute neutrophil count, ANC $\geq 0.5 \times 10^9/L$) and for platelets (PLT; i.e. the time to achieve a PLT count $\geq 20 \times 10^9/L$ unsupported by transfusion) were recorded. The study was approved by the Institutional Review Board.

Graft composition

We retrospectively examined 146 PBSC apheresis products. After collection the following parameters were recorded: White Blood Cell (WBC), neutrophil, PLT and CD34+ cell concentration. Within one month from freezing, post-thawing cell viability of Total Nucleated Cells (TNC) and CD34+ cells was assessed and the actual dose of transplanted CD34+ cells was calculated. WBC, neutrophil and PLT concentrations at collection were evaluated using an automated

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blood cell counter (ADVIA2120, Siemens, Germany). CD34+ cell concentration was assessed according to the single platform-ISHAGE protocol [11]. TNC and CD34+ cell viability were evaluated by 7-AAD staining on a satellite vial [11]. DMSO10% and albumin 5% were chosen for cryopreservation with a final TNC concentration $<3 \times 10^8/\text{mL}$.

Statistical analysis

The correlation between different variables was analysed by the Spearman's rank nonparametric test; differences between continuous variables were analyzed by the Mann-Whitney U test and differences between categorical variable were analyzed by the χ^2 test. The p values <0.05 were regarded as statistically significant.

Results and Discussion

The dose of CD34+ cells infused is the main predictor of haematopoiesis recovery

In our series of patients the median engraftment times were 11 days (range 8-31) for neutrophils and 12 days (range 8-35) for PLTs. On the whole, a median dose of $4.85 \times 10^6/\text{kg}$ viable CD34+ cells per transplant was given (range 1.98-36.97). As previously reported [3-9], the length of recovery was inversely correlated to the dose of infused CD34+ cells, for both neutrophils and platelets ($p<0.001$ for both, Figure 1). We did not

detect any correlation between engraftment times and concentrations of total WBCs, neutrophils and PLTs in the grafts. These results are apparently discrepant from previous reports of delayed engraftment for PBSC products containing high dose of neutrophils [7-9]. Nevertheless, we emphasize that our data refer to neutrophil concentrations in the bags, and not to the total neutrophil content in the grafts, so that a direct comparison is not appropriate.

Graft composition influences post-thawing viability

Data gathered in both allogeneic and autologous hematopoietic stem cell transplantation, suggest that low post-thawing cell viability predicts delayed neutrophil engraftment [2,7,12]. Indeed, we investigated this issue in our series of patients. The median values of post-thawing viability were 71.4% (range 28.1-94.3) for TNC and 95% (range 48.3-99.9) for CD34+ cells. These parameters were reciprocally strongly correlated ($r=0.409$, $p<0.001$). When patients were grouped according to the median values of post-thawing viability, a weak but significant trend for an extended time for engraftment of neutrophils and platelets was observed in patients who had received products with CD34+ cell viability lower than 95% (Figure 2). These effects could not be ascribed to differences in underlying diseases or age at transplants. Regarding the grafts' characteristics, we observed that PBSC products with lower CD34+ cell viability had higher PLT concentrations than

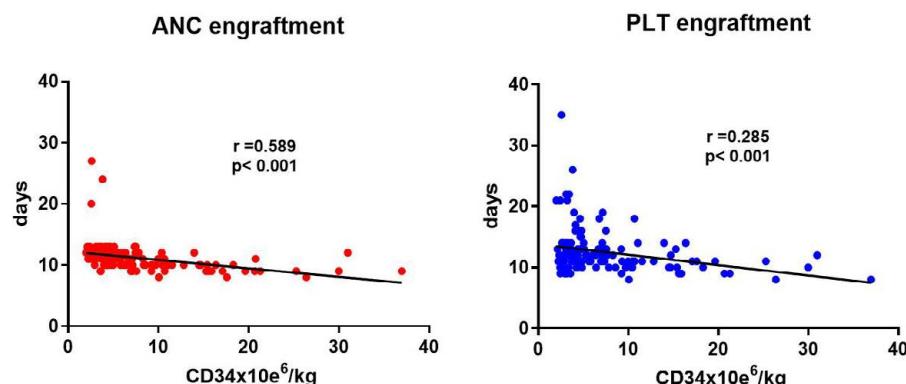


Figure 1: All nucleated cell (ANC) and platelet (PLT) engraftment times according to the CD34+cell dose infused.

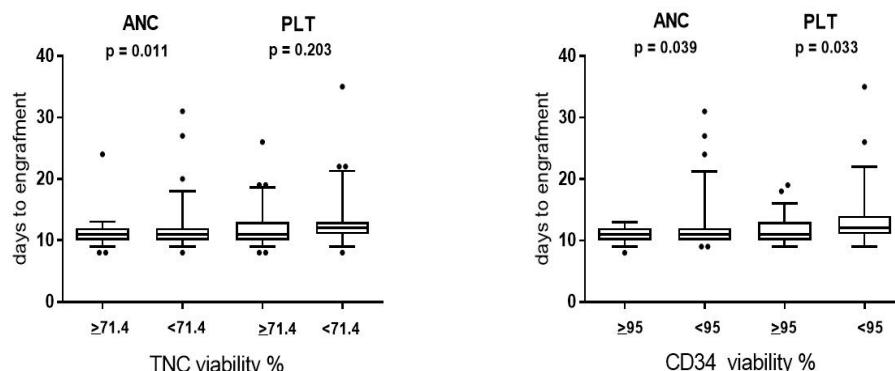
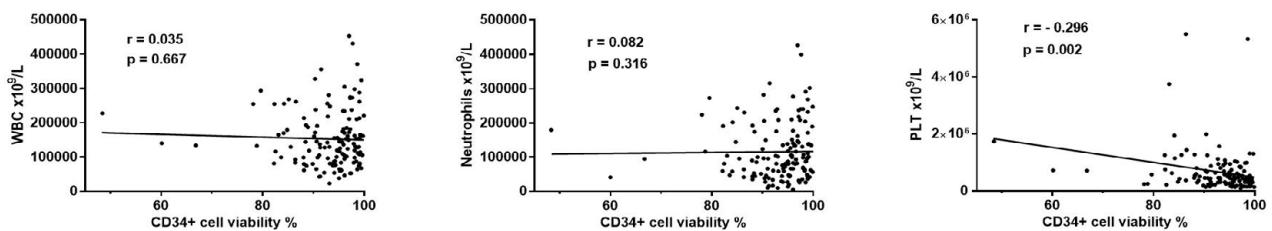


Figure 2: Effect of graft composition on hematopoietic recovery. Peripheral blood stem cell products were grouped according with median values of TNC and CD34+ cell viability obtained from a series of 146 transplants.

	CD34+ cell viability <95% (n=70)	CD34+ cell viability ≥ 95% (n=76)	p value
Patients characteristics			
Sex (male/female)	45/25	39/37	0.078
Diagnosis (NHL/MM/HL)	28/33/9	31/34/11	0.101
Age at transplantation (years)	53 (20-67)	57 (25-66)	0.055
CD34+ cell dose ($\times 10^6/\text{kg}$)	4.4 (1.9-36.9)	5.2 (2.1-30.9)	0.147
Neutrophil engraftment (days)	11 (9-31)	11 (8-13)	0.039
Platelet engraftment (days)	12 (9-35)	11 (9-19)	0.033
Graft characteristics			
WBC $\times 10^9/\text{L}$	131.095 (23.130-356.200)	143.510 (46.320-45.3240)	0.414
Neutrophils $\times 10^9/\text{L}$	90.125 (99.22-315.593)	102.413 (47.700-426.498)	0.294
PLT $\times 10^9/\text{L}$	563.500 (137.000-5507.000)	372.000 (96.000-533.7000)	<0.0001

Table 1: Patient and graft characteristics in 146 ASCT procedures, grouped according to the post-thawing CD34+ cell viability. NHL, non-Hodgkin lymphoma; MM, multiple myeloma; HL, Hodgkin lymphoma.

A



B

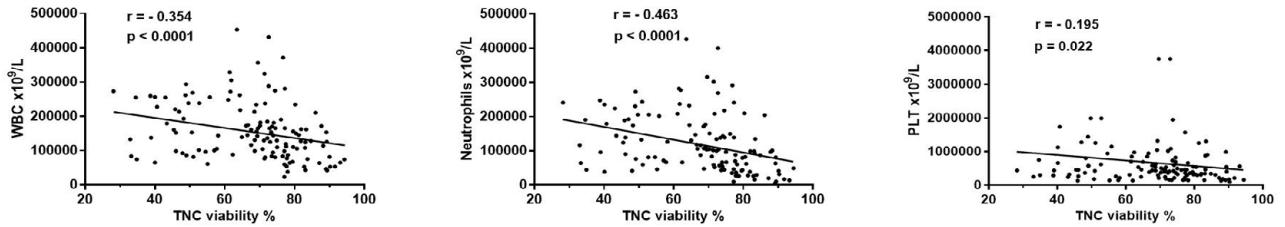


Figure 3: Characteristics of peripheral blood stem cell products and post thawing viability. A) Correlation with CD34+ cell viability; B) Correlation with total nucleated cells (TNC) viability. The median values of parameters in 146 PBSC products were: WBC $135.950 \times 10^9/\text{L}$ (range 23.130-453.240); PLT $457.000 \times 10^9/\text{L}$ (range 9.600-550.7000); Neutrophil $95.775 \times 10^9/\text{L}$ (range 47.710-426.499).

others (Table 1 and Figure 3A). Similarly, patients receiving APBS products with TNC viability lower than 71.4% had longer time to neutrophil engraftment (Figure 2). These PBSC products had higher concentrations of WBC, neutrophils and PLTs (Table 2 and Figure 3B). On the whole, our findings regarding the negative association between low TNC viability and delayed neutrophil engraftment reinforce previously published data with similar results [7,12]. However, in our patients, the low CD34+ cell viability appears relevant for both neutrophil and PLT recovery. In this regard, Castellano et al., recently reported that high TNC concentrations negatively impact on CD34+ cell viability; nevertheless, in this study, the authors did not include among the analyzed parameters the PLT concentration [13].

High PLT concentration in PBSC products is detrimental for cell viability

To our knowledge, the negative impact of high PLT count on progenitor cell viability has not previously reported. Therefore, we

attempted to confirm this findings by evaluating the relationship between these parameters (PLT concentration and CD34+ cell viability) in experimental conditions. We split two cord blood units in 18 overall aliquots: in 16 of them, variable amounts of ABO-matched apheresis PLTs were added, in order to achieve cell samples with progressively increasing PLT concentrations (from basal values to $2.000.000 \times 10^9/\text{L}$). Cell suspensions were then cryopreserved and frozen, according with procedures normally used for PBSC product samples. After few days, vials were thawed and viability tests were performed. We could confirm that a progressive decrease of CD34+ cell viability occurs along the increase of PLT concentration (Figure 4). During degranulation, PLTs release a myriad of pro-inflammatory mediators; in addition, thawed samples could contain a load of PLT microparticles, bound to residual PLT membranes [14]. Therefore, it is conceivable that all these products may be also released from PLT destroyed during the PBSC thawing, accounting for the decreased cell viability in samples highly

	TNC cell viability <71.4% (n=78)	TNC cell viability ≥ 71.4% (n=68)	p value
Patients characteristics			
Sex (male/female)	49/29	35/33	0.178
Diagnosis (NHL/MM/HL)	21/47/10	38/20/10	0.005
Age at transplantation (years)	56 (21-67)	55 (20-66)	0.941
CD34+ cell dose ($\times 10^6/\text{kg}$)	4.6 (1.9-30.9)	5.2 (2.1-36.9)	0.147
Neutrophil engraftment (days)	11 (8-31)	11 (8-24)	0.012
Platelet engraftment (days)	12 (8-35)	11 (8-26)	0.236
Graft characteristics			
WBC $\times 10^9/\text{L}$	155.110 (54.350-453.240)	113.900 (23.130-431.050)	<0.0001
Neutrophils $\times 10^9/\text{L}$	120.725 (33.479-426.498)	66.515 (47.700-399.583)	<0.001
PLT $\times 10^9/\text{L}$	563.500 (137.000-5507.000)	372.000 (96.000-533.7000)	<0.0001

Table 2: Patient and graft characteristics in 146 ASCT procedures, grouped according to the post-thawing TNC cell viability. NHL, non-Hodgkin lymphoma; MM, multiple myeloma; HL, Hodgkin lymphoma.

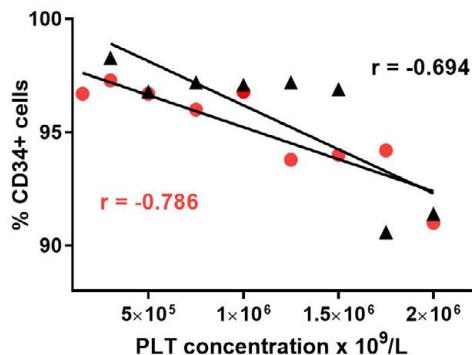


Figure 4: Effect of increasing concentrations of PLTs on CD34+ cell viability. Several aliquots of two cord blood units were added with increasing amounts of ABO-matched apheresis platelets, in order to achieve cell samples with progressively increasing PLT concentrations.

contaminated by PLTs. Importantly, these findings suggest that either PLT removal before freezing, or post-thawing washing, might prevent viable CD34+ cell loss, and also have an indirect positive effect on the hematopoietic recovery.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Contribution

CGV and MB involved in conception and design, data collection, analysis and interpretation, apheresis procedures, manuscript writing, and final approval of manuscript; NO and MGI involved in data collection, viability experiment conductions and final approval of manuscript; FA involved in data collection and analysis; SS involved in patient care, data collection and final approval of manuscript; GZ and NP involved in conception and design of the study and final approval of manuscript; VDS and LT involved in conception and design, analysis and interpretation of data, manuscript writing, and final approval of manuscript.

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