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Unraveling stem cell and progenitor subsets in autologous grafts according to methods of mobilization: implications for prediction of hematopoietic recovery

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Abstract

Background aims. In the autologous setting, granulocyte colony-stimulating factor (G-CSF) (G), or, when failing, G plus plerixafor (G+P), are common regimens for mobilization of stem cells into peripheral blood. To delineate mobilization effects on graft composition and hematopoietic recovery, we compared contents of stem cells and progenitor cells in products of G+P- and G patients. Paired samples of G+P patients and prior insufficient G mobilization were available for analyses. *Methods.* Subset analyses of grafts were performed by flow cytometry and myeloid colony-forming assay. In search of new markers to ascertain graft quality, we determined the fractions of aldehyde dehydrogenase bright ($ALDH^{br}$) cells. *Results.* G grafts contained higher percentages of CD34+ cells, CD34+CD38- cells, and committed progenitors (CD34+CD38+) compared with G+P grafts. A detailed characterization of the mobilized CD34+ cell subset showed higher percentages of CD38− among the CD34+ cells of the G+P group ($P = 0.032$). In contrast, the CD34+ cell subset in G grafts was characterized by a higher percentage of $ALDH^{br}$ cells ($P < 0.0001$). Studying engraftment and day +100 graft function the G and G+P transplanted patients were comparable with respect to neutrophils, whereas in platelets they differed. In the prediction of engraftment and hematopoietic recovery, the dose of infused $ALDH^{br}$ cells correlated best to both platelet ($r = 0.565$, $P = 0.002$) and neutrophil reconstitution ($r = 0.366$, $P = 0.06$). *Conclusions.* Besides showing dissimilar distributions of CD34+CD38− cells and progenitors in G and G+P grafts, this study further designated $ALDH^{br}$ as a promising marker in determination and prediction of graft quality and hematopoietic recovery.

Key Words: *aldehyde dehydrogenase, G-CSF, hematopoietic stem cell transplantation, plerixafor*

Introduction

High-dose chemotherapy supported by autologous hematopoietic stem cell transplantation (AHSCT) plays a pivotal role in the treatment of patients with multiple myeloma (MM) and chemosensitive, relapsed or refractory lymphoma (1). In this setting, the quantity of CD34+ cells has been used as a surrogate marker for the number of hematopoietic stem cells (HSCs) in grafts and is considered the determining factor of neutrophil and platelet engraftment (2,3). Chemotherapy followed by granulocyte colony-stimulating factor (G-CSF) or G-CSF alone have been the standard methods (G) for mobilizing CD34+ cells into peripheral blood stem cells (PBSCs) (4). Inadequate mobilization is seen in 5–30% of patients mobilized on these standard regimens, which invokes the need of alternative mobilization strategies (5,6). Plerixafor is a reversible and selective antagonist of the CXCR4 chemokine receptor that blocks CXCR4 and stromal cell–derived factor-1-α interactions, thus causing release of HSCs and progenitors from the bone marrow (BM) niche. Hence, in patients failing or likely to fail G mobilization, G-CSF plus plerixafor (G+P) increases the yield of CD34+ cells (3,7,8).

The method of mobilization may affect the numbers and characteristics of HSC and progenitor subsets in grafts (1,9). Indeed, extended characterization and subtyping of these cell subsets may address pertinent issues regarding graft quality in terms of engraftment and hematopoietic recovery.

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In a *post hoc* analysis of phase III trials, it has been reported that CD34+ cell dose did not correlate with the time to neutrophil or platelet engraftment, long-term neutrophil or hemoglobin recovery or patient survival (2,3,10). The dissociation between CD34+ cell content and graft failure in individual patients may be explained by the nature of HSC and progenitor subsets mobilized (4,11,12).

Marking of aldehyde dehydrogenase (ALDH) activity, a cytosolic enzyme and a functional marker with bright expression ($ALDH^{br}$) in HSCs and progenitors, may confer a complementary strategy to CD34+ cell enumeration in graft quality evaluation (5,6,13–15). The quantity of $ALDH^{br}$ cells in grafts has been shown to correlate with hematopoietic reconstitution after AHSCT, after G mobilization (3,7,8,16,17). However, the role of ALDH marking in G+P-derived as compared with G-derived grafts remains unsettled. We compared G- and G+P-mobilized grafts, measuring HSC-enriched and progenitor subsets with the use of CD34, CD38 and ALDH for evaluation of graft quality. To further designate the impact of mobilization method on graft content, in a small patient cohort we were able to perform paired analyses of PBSCs from insufficient G mobilization and the subsequent successful G+P mobilization.

Methods

Patients

Sampling was performed after informed consent was obtained. The study was conducted in accordance with the *Declaration of Helsinki* and approved by the local ethics committee. Twenty-two patients with either MM or lymphoma were treated from March 2009 to March 2011 with G+P as part of PBSC mobilization after failing G mobilization, either because of low CD34+ cell number before apheresis or low CD34+ cell collection yield ($<2 \times 10^6$ CD34+/kg). A control group of patients (n = 22) was matched with respect to diagnosis, sex, age, priming and conditioning treatment, number of treatment lines before AHSCT and radiation therapy. The demographic and clinical characteristics of patients included are summarized in Table I. In the G group, five patients were not given transplantation, one patient died before day +100 and one patient was admitted to AHSCT at another institution. In the G+P group, five patients were not transplanted, two patients died before day +100 and one patient was transferred to AHSCT at another institution. Patients not undergoing AHSCT were predominantly diagnosed as having follicular lymphoma, and PBSCs were collected for possible later relapse. Eight patients from the G+P group had cryopreserved cells available from an insufficient PBSC collection after G mobilization (G_i). This allowed for paired analyses of G_i samples with subsequent successful G+P mobilization samples (G_i+P).

Mobilization procedures and apheresis

With the exception of three patients, all were mobilized by a combination of chemotherapeutic priming followed by filgrastim 12 μg/kg per day starting 4 d before the projected 1st day of apheresis and continuing until the required CD34+ cell target dose (Table I) was obtained. Leukocyte differential count and PB CD34+ cell enumeration were performed daily at 6:00 AM until completion of apheresis or observation of mobilization failure. Apheresis was commenced at CD34+ cell counts $\geq 10 \times 10^3$/μL. In the case of poor mobilization or low collection yield, 12 h after first filgrastim administration, the patients received a dose of filgrastim (12 μg/kg) followed by a plerixafor injection (240 μg/kg per day) at 10 PM (G+P and the G_i+P groups).

Collection of PBSCs was performed with a COBE Spectra, Software Version 6.1 (CaridianBCT, Lakewood, CO, USA). Acid-citrate-dextrose formula A was used as the anticoagulant. The minimum volume of blood processed to achieve 2×10^6 CD34+ cells/kg was assessed by use of the formula: 6.5 × patient weight (kg)/preharvest CD34+ cells per μL peripheral blood. The median processed volume for G patients was 12.5 L (range, 9.6–33.8 L) and for G+P patients was 18.6 L (range, 12–37.8 L). The collect flow rate used was 1 mL/min and the inlet volume was 2.3 × total blood volume. Daily apheresis continued until a minimum target collection was met. The PBSCs were cryopreserved in 10% dimethylsulfoxide through the use of a controlled rate freezer and stored in liquid nitrogen (18).

Conditioning, reinfusion of PBSCs and post-transplantation course

All patients with MM were conditioned with melphalan (200 mg/m^2), and most patients with lymphoma were conditioned with BEAM (carmustine 300 mg/m^2 on day −6; etoposide 200 mg/m^2 on days −5 to −2; cytarabin 200 mg/m^2, and melphalan 140 mg/m^2 on day −2). Four patients with lymphoma received TBI-Cy (total body irradiation; total dose, 12.5 Gy on days −8 to −5, followed by cyclophosphamide 60 mg/kg on days −4 and −3) and one patient was treated with Z-BEAM: (Zevalin, ^{90}Y-ibritumomab tiuxetan 15.0 MBq, 0.4 mCi/kg plus BEAM). Pre-freeze reinfused CD34+ cells were counted as