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Storage Duration of Autologous Stem Cell Preparations Has No Impact on Hematopoietic Recovery after Transplantation

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A B S T R A C T

Peripheral blood stem cells (PBSCs) are widely used for autologous blood stem cell transplantation (ABSCT). These cells must be stored for months or even years, usually at temperatures ≤–140°C, until their use. Although several in vitro studies on $CD34^+$ viability and clonogenic assays of PBSCs after long-term storage have been reported, only a few publications have investigated the influence of long-term storage on in vivo hematopoietic reconstitution. In this study, we retrospectively analyzed hematopoietic recovery after storage of PBSCs via controlled-rate freezing (CRF) and cryostorage in 10% DMSO at ≤–140°C in 105 patients with multiple myeloma who received high-dose melphalan before ABSCT. Three groups of PBSC transplantation (n = 247) were delineated based on the storage period: short-term (≤12 months, n = 143), medium-term (>12 and ≤60 months, n = 75), and long-term storage (>60 months, n = 29). A neutrophil increase of ≥.5 × 10^9/L in medium-term or long-term PBSC cryopreservation groups was observed at day 14 after ABSCT; this increase was comparable to patients who received briefly stored PBSCs (day 15). No negative effect of PBSC storage duration was observed on leucocyte or neutrophil reconstitution. Platelet reconstitutions of ≥20 × 10^9/L and 50 × 10^9/L were observed after median times of 10 to 11 and 13 to 14 days after ABSCT, respectively. No influence of PBSC storage duration on platelet recovery of ≥20 × 10^9/L and ≥50 × 10^9/L was observed in the 3 storage groups ($P = .07$, $P = .32$). The number of previous ABSCTs also had no significant impact upon hematopoietic reconstitution. In conclusion, these results indicate that long-term cryopreservation of PBSC products at vapor nitrogen temperature after CRF does not have a negative effect on hematopoietic recovery even after prolonged storage.

INTRODUCTION

High-dose (HD) chemotherapy followed by autologous blood stem cell transplantation (ABSCT) represents a standard therapy for a variety of malignant diseases, including multiple myeloma (MM) [1]. Peripheral blood stem cells (PBSCs) have become the most widely used source for hematopoietic stem cells in this setting [2,3]. Single ABSCT was initially established for MM first-line therapy [4,5], but subsequent randomized clinical trials demonstrated a benefit in overall survival of a tandem ABSCT compared with that of a single ABSCT, especially in MM patients not achieving at least partial remission after the first transplantation [6,7]. Later studies revealed that salvage ABSCT is an effective treatment option for MM patients who relapse after a sustained remission of >1 year after a prior ABSCT [8-10]. Considering the difficulties of collecting additional PBSC grafts after the initial myeloablative HD chemotherapy and ABSCT [11], many centers collect up to 3 PBSC grafts before the first ABSCT to maintain the option of another ABSCT in case of relapse.

This treatment schedule implies that PBSC products need to be stored for months or even years until use. Although alternative simplified methods of cryopreservation in mechanical uncontrolled-rate freezers at ≤–80°C are described in the literature [12-14], PBSCs are usually subjected to controlled-rate freezing (CRF) and stored at liquid or vapor phase nitrogen temperature (≤–130°C) for long-term periods with cryoprotectants, such as dimethyl sulfoxide (DMSO)

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[15,16]. Several studies have shown that CFR-cryopreserved PBSC products maintain CD34+ viability, function, and engraftment potential even beyond 10 years [17-22]. However, the limitations of these studies include an absence of evidence for in vivo PBSC engraftment in humans [17,18,20,21], a relatively low number of patients, and heterogeneous disease entities [19,22,23].

The aim of this study was to evaluate the influence of medium-term and long-term PBSC storage on hematopoietic reconstitution of sequential ABSCTs in a large homogenous group of MM patients who received HD melphalan as a conditioning regimen.

PATIENTS AND METHODS

Patient Selection and Data Matching

A retrospective single-center analysis of MM patients who underwent HD melphalan chemotherapy and ABSCT between September 2003 and October 2015 at our university hospital was performed. Patients were included in the evaluation only if 1 of the ABSCTs in these patients included PBSCs with a storage duration >12 months. Clinical parameters (gender, age), Salmon and Durie stage at first diagnosis, type of monoclonal protein, modality of induction and mobilization therapy, PBSC collection result, remission status before and after each ABSCT, transplanted CD34+ cell number, and hematological reconstitution data were collected retrospectively and evaluated with regard to PBSC storage duration before ABSCT (storage group A: ≤12 months; storage group B: >12 and ≤60 months; storage group C: >60 months). Retrospective data analysis was approved by the ethics committee of the medical faculty, Heidelberg University.

PBSC Mobilization and Collection

A cyclophosphamide, doxorubicin, and dexamethasone (CAD; cyclophosphamide 1000 mg/m^2/day on day 1, doxorubicin 15 mg/m^2/day on days 1 to 4, dexamethasone 40 mg/day orally [p.o.] on days 1 to 4) chemotherapy regimen was used for mobilization. Granulocyte colony–stimulating factor, 5 μg/kg to 10 μg/kg bw per day) was injected subcutaneously starting 5 days after completion of CAD chemotherapy until the end of PBSC collection. When leukocytes reached ≥5.0×10^9/L, the number of peripheral blood CD34+ cells was determined by flow cytometry as previously described [24]. Leukapheresis was initiated when peripheral blood CD34+ cell count reached ≥20/μL. Stem cell collection was performed using the COBE Spectra apheresis machine (MNC program, software version 4.7 or 6.1; Terumo BCT, Garching, Germany). Beginning in March 2013, the Spectra Optia apheresis machine (MNC program, software version 7.2 and 11.2) was increasingly used in parallel with the COBE Spectra apheresis machine. As described previously, all of these apheresis systems had a similar collection efficiency [25]. The collection was restricted to a maximum of 5 hours per leukapheresis session. Acid citrate-dextrose was used at an inlet:anticoagulant ratio of 11:1 to 15:1 with an inlet flow rate of 40 mL/minute to 100 mL/minute. The collection flow rate was set at 1 mL/minute. Continuous calcium gluconate infusion (1 mmol/hour) was used to prevent side effects of citrate. The minimum number of CD34+ cells for 1 transplant was defined as ≥2.0×10^6/kg body weight (bw), with the goal of collecting enough CD34+ cells for 3 transplantations.

PBSC Cryopreservation

PBSCs were processed and stored in accordance with the German Medical Council and responsible scientific society's guidelines [26-28]. Collected cells were usually stored for 24 to 48 hours at 2°C to 6°C until cryopreservation, with a maximum nucleated cell (NC) concentration of 2×10^8/mL. After storage, PBSC products were centrifuged if necessary and diluted with autologous plasma or resuspension medium (Plasmalyte A, Baxter, Unterschleissheim, Germany or Composol PS, Fresenius Kabi, Bad Homburg, Germany) and CryoSure-DMSO (WAK-Chemie Medical, Steinbach, Germany) to obtain a target NC concentration of ≤5×10^8/mL and a total volume of 100 mL per bag. The final product included 10% DMSO and was stored in Cryocyte bags (Baxter, Unterschleissheim, Germany or CryoMACS freezing bags (Miltenyi, Idarobrstein, Germany). Cells were subjected to CRF (Biofreeze BV50, Consarctic, Schoellkrippen, Germany) and stored in vapor phase nitrogen at a temperature of <–140°C. At the time of ABSCT, cryopreserved bags were thawed at the bedside in a plasmatherm (Barkey GmbH & Co. KG, Leopoldshoehe, Germany) at 37°C, and PBSCs were reinfused within a maximum of 10 minutes of thawing using standard transfusion filters without previous washing for purposes of DMSO depletion.

PBSC Quality Assessment

PBSC product quality assessment was performed at different time points of processing and storage. A volume determination, an enumeration of NC and red blood cells, and flow cytometry–based CD34+ cell quantification were performed directly after PBSC collection in accordance with the Stem Cell Enumeration Committee Guidelines of the International Society for Cell Transplantation [29]. Shortly before freezing, a microbiological culture sample was obtained. NC enumeration and NC viability measurement by propidium iodide exclusion were performed from PBSC aliquots after freezing and once again in case of a storage duration >24 months. Overall, the following target values were defined for the end product (1 PBSC transplantation): a total volume of 100 mL per portion (up to 3 portions possible), NC concentration ≤5×10^8/mL, CD34+ cell number ≥2×10^6/kg bw, red blood concentration ≤.1 mL/mL, no microbial growth, and minimum NC viability of 50%. Viability testing was valid for a maximum duration of 3 years before it had to be repeated before transplantation.

HD Chemotherapy, ABSCT, and Sequential Grafts

Clinical indication and eligibility for HD melphalan and ABSCT were determined by the treating physician. All patients received HD melphalan (100 mg/m^2, day –3 and day –2, 1-hour infusion) as a conditioning regimen. Melphalan dosage was reduced by 50% when creatinine clearance was ≤40 mL/minute (n = 2). For prevention of chemotherapy-induced nausea and vomiting, an intensified oral supportive medication regimen (dexamethasone 4 mg day –3; dexamethasone 2 mg day –2 to day –1, granisetron hydrochloride 2 mg days –3 to day 0, aprepitant 125 mg day –3, aprepitant 80 mg day –2 to day 0) was used [30]. A minimum of 2.0×10^6 CD34+ cells/kg patient body weight was reinfused on day 0 using standard supportive therapy (500 mg acetaminophen p.o., 2 mg clemastine i.v., 10 mg dihydrocodeine p.o.). As antibiotic and antiviral prophylaxis, patients received daily ciprofloxacin 2×500 mg p.o. until hematological reconstitution and acyclovir 2×400 mg p.o. for 6 months after ABSCT. No growth factors were used after transplantation. Sequential HD chemotherapy and ABSCT were performed as a part of first-line therapy if a complete remission or near complete remission was not reached after the first round of HD melphalan and ABSCT. Even if any future autografts have been dismissed, the patients received only the CD34 cells amount of 1 transplantation (no reinfusion of remaining transplantations).

Assessment of Remission Status and Hematological Reconstitution

The remission status was assessed according to international uniform response criteria for multiple myeloma [31]. After ABSCT, a blood count was performed on a daily basis until leucocyte/neutrophil and platelet engraftment. *Leucocyte engraftment* was defined by a leucocyte count of ≥1.0×10^9/L. The number of days with leucocytes <1.0×10^9/L was recorded as days in aplasia. *Neutrophil recovery* was defined as the first of 3 consecutive days on which neutrophils reached ≥.5×10^9/L. *Platelet engraftment* was defined as the first day on which platelets reached ≥20×10^9/L without platelet transfusion. Because in some patients the platelet count did not drop under 20×10^9/L or was not assessable because of platelet transfusion, we also calculated days until platelets ≥50×10^9/L as a second variable for platelet engraftment.

Statistical Analysis

Descriptive statistics and comparisons between groups were performed by R studio 7.6. Data are presented as absolute numbers and percentage if not otherwise stated as median and range. For comparison of categorical variables (HD-melphalan dose modification, platelets not <20×10^9/L, and platelet transfusion), Freeman-Halton extension of Fisher's exact test was used. To identify differences among group means (age at ABSCT; transplanted CD34+ cell number; PBSC storage duration; days to engraftment of leucocytes, neutrophils and platelets; and days in aplasia), comparisons of more than 2 groups were performed via analysis of variance, and comparisons between 2 groups were performed with a 2-tailed Student's *t*-test. Leucocyte, neutrophil, and platelet recovery over time were calculated and plotted using Kaplan-Meier survival analysis. To calculate differences between engraftment curves, a log-rank test was used. $P < .05$ was considered statistically significant. To analyze the relationship between the number of transplanted CD34+ cells and time to hematological engraftment, a linear regression approach was applied. The goodness-of-fit of the linear regression is provided by the coefficient of determination R^2. $R^2 \geq .8$ was considered to indicate a relevant relationship.

RESULTS

Patient Characteristics

Between 2003 and 2015, data from 105 MM patients (72 male and 33 female) were analyzed. The median age at first diagnosis and PBSC collection was 57 (range, 33 to 71) years. Ninety-six (91%) patients had a Salmon and Durie stage IIIA at first diagnosis. The majority of patients (n = 52, 49%) received bortezomib, doxorubicin, and dexamethasone (PAD)