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Assessment of immune reconstitution status in recipients of a successful hematopoietic stem cell transplant from peripheral blood after reduced intensity conditioning

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ABSTRACT

Objective: To document immune reconstitution status after hematopoietic stem cell transplantation (HSCT) for malignant hematologic diseases.
Methods: Hematology patients who received a reduced intensity conditioning (RIC) were followed after successful allogeneic or autologous HSCT. Patients had at least 100 days post-transplant. T, B and NK cells in peripheral blood (PB), and CD34+, CD133+ progenitor cells in bone marrow (BM) and peripheral blood (PB) were determined by flow cytometry.
Results: Twenty-seven HSCT recipients, 19 allogeneic and 8 autologous, were studied at a median 155 (100–721) days post-transplant. In the whole group the median value of CD34+ cells was 1.03% in the bone marrow and 0.04% in PB, whereas values for CD133+ cells were 0.39% and 0.13%, respectively, without statistical differences between autologous and allogeneic recipients. Significantly more B cells (CD3−/CD56−/CD19+) were found in the autologous compared to the allogeneic group, 12.6 vs. 5.01, $p = 0.04$. An increased number of CD8+ lymphocytes with a 0.63 CD4:CD8 relationship was documented in PB.
Conclusion: In clinically recovered autologous and allogeneic HSCT recipients BM and PB CD34+/CD133+ hematoprogenitor homeostasis is maintained within normal ranges, with better B-cell reconstitution in the autologous group.

1. Introduction

The success of hematopoietic stem cell transplantation (HSCT) for the treatment of hematologic malignancies is dependent on efficient eradication of the malignant clone and on successful reconstitution of the host's hematopoiesis and immune system [1], the feasibility of CD34+ selected allografting and a low incidence of severe graft-versus-host disease. The CD34+ cell fraction in bone marrow (BM), peripheral blood (PB) and umbilical cord blood (UCB) is remarkably heterogeneous and contains diverse subsets of hematoprogenitor cells at variable degrees of lineage commitment [2]. Recently, the potential importance of determining CD133+ cell content in the graft as a quality control step has been documented and it allows for a more precise discrimination and quantification of hematoprogenitors [3].

On the other hand, innate immune recovery is observed during the few first months after HSCT, with monocytes being followed by granulocytes and natural killer (NK) cells. Complete reconstitution of adaptive immunity, consisting of T (cellular) and B (humoral) lymphocytes, can take 24 or more months. During the first post-HSCT months the principal mechanism for T-cell recovery is peripheral expansion of donor cells, leading at this stage to clonal expansion of CD8+ T lymphocytes with a restricted repertoire and delayed or incomplete immune reconstitution has been associated with significant morbidity and mortality, especially after allogeneic HSCT due to infections and disease relapse [4].

T cell immunity is particularly affected by diverse factors including, but not limited to, type of conditioning regimen, thymic involution in the patient, type and source of graft, stem cell dose, donor–host disparity, GVHD prophylaxis and disease, as recently reviewed [5]. On the other hand, B cells recover at about six and nine months after auto and allo-HSCT, respectively [6].

Over the last years hematopoietic grafting at our reference center has been performed employing a RIC protocol, with good results [7–9]. Reduced-intensity conditioning regimens cause less damage and less graft versus host disease (GvHD) than total irradiation regimens [10], allowing for this important therapeutic intervention to be carried out at low cost and in older individuals. Although diverse aspects of

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hematopoietic grafting have been studied, other remain to be completely elucidated, chief among these is the characterization of immune system reconstitution at the functional level, and in this respect there is scarce information documenting the relationship between peripheral and bone marrow lymphocyte subset recovery in the context of reconstitution in patients who received a HSCT after being conditioned with RIC schemes. The steady state post-HSCT in PB and BM status of hematoprogenitor cells, defined as those expressing CD34 and/or CD133 immune markers, as well as the relationship between this two compartments has not been defined at this stage in this hematologic population.

The purpose of this study was to assess the status of immune cell reconstitution, the relationship between peripheral and bone marrow lymphocyte populations after HSCT and their relationship with clinical outcome in hematologic patients receiving a RIC regimen.

2. Material and methods

2.1. Patients

Twenty-seven clinically asymptomatic patients attending the Hematology Department at the "Dr. José E. Gonzalez" University Hospital of the School of Medicine of the Autonomous University of Nuevo León, in Monterrey, México, who had passed day +100 after receiving an allogeneic or autologous HSCT for diverse malignant or pre-malignant hematologic diseases who accepted to participate in the study and give a bone marrow biopsy specimen for analysis were included. Informed consent was obtained. Patients who suffered a relapse and those who did not sign the consent form were excluded. The Institutional Review Board and the Ethics Committee approved the protocol of the study.

2.2. Conditioning regimen, transplant procedure and follow-up

Briefly, the administered RIC conditioning consists of a scheme with dosage based on ideal weight, which includes busulphan 4 mg/kg p.o. on days −6 & −5; cyclophosphamide 350 mg/m^2 once daily i.v. on days −4, −3 & −2; fludarabine 30 mg/m^2 i.v. once daily on days −4, −3 & −2; cyclosporine A (CyA) 5 mg/kg p.o. starting on day −1; and methotrexate 5 mg/m^2 i.v. on days +1, +3, +5 & +11. CyA is continued through day 180, with adjustments to obtain serum CyA levels of 150–275 ng/ml, and then tapered over 30–60 days [8]. If GvHD developed, CyA was tapered over longer periods. Ondansetron 1 mg i.v. every hour over 4 h after chemotherapy, an oral quinolone and an azole were used in all patients until granulocytes were $>0.5 \times 10^9$/L.

All donors were matched-related siblings, and were stimulated with 10 μg/kg of G-CSF subcutaneously for five days prior to CD34+ cell automated collection [7] CD34+ cells at a dose $\geq 2.5 \times 10^6$/kg of body weight were infused on day 0. Afterwards, patients were closely followed to document time to neutrophil and platelet recovery to ≥500/μL and ≥20,000/μL, respectively. Engraftment was also assessed by chimerism analysis by flow cytometry and clinical follow-up was carried out on an outpatient basis. Transplants in our center are performed as an outpatient procedure, in order to make this modality of therapy financially viable and to reduce the risk of hospital acquired post-transplant infections [7,8].

2.3. Phenotypic analysis by multiparameter flow cytometry

For each donor's samples of bone marrow or peripheral blood, individual blood count with a Sysmex XS-1000i (Lincolnshire, IL, USA) was performed. For antibody staining, 1×10^6 WBC were suspended in a total volume of 100 μL containing phosphate-buffered saline (PBS), (Miltenyi Biotec) with 0.5% BSA (bovine serum albumin) and 0.09% sodium azide (BD Biosciences) in BD Falcon round-bottom tubes (BD Biosciences) and incubated for 15 min in the dark.

Afterwards the samples were stained with anti-CD8 FITC (clone SK1, BD Biosciences), anti-CD56 PE (clone MY31, BD Biosciences), anti-CD45-V500 (clone 2D1, BD Biosciences), anti-CD4 V450 (clone RPA-T4, BD Biosciences), anti-CD19 APC-H7 (clone SJ25-C1, BD Biosciences), anti-CD45 FITC ES (clone 2D1, BD Biosciences), anti-CD34 APC (clone 8G12, BD Biosciences), anti-CD3 PerCP (clone UCHT1, DAKO), anti-CD133 PE (clone 293C3, Miltenyi Biotecnology) and then incubated for 15 min in the dark at room temperature. After incubation, the cells were suspended with 2.0 mL of a 1/10 dilution FACSLysing solution (BD Biosciences) and incubated in the dark for 10 min at room temperature. Cells were centrifuged at 540 g × 5 min, the supernatant was discarded using Pasteur pipettes and the cell pellet was suspended in 50 μL of buffer solution. The cells were washed with 2.0 mL of PBS containing 0.5% BSA and 0.09% sodium azide, vortexed, and centrifuged at 540 g × 5 min. Finally, the supernatant was discarded and the cells were suspended in 200 μL of PBS containing 0.5% BSA. Cells were kept at 4 °C before analysis. Dead cells and debris were discarded by using Forward scatter/Side scatter (FSc/SSc) exclusion in the sample dot plots, and the main location of lymphocytes and monocytes was considered for the gating strategy. All samples were analyzed in a FACSCanto II cytometer (BD Biosciences).

2.4. Statistical analysis

For data analysis, SPSS v. 20.0 statistical package (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) was used. Patients were analyzed as a whole and then segregated by type of transplant in autologous and allogeneic groups. Descriptive analysis was performed, obtaining means and standard deviation, medians and ranges according to the variable distribution calculated with Kolmogorov–Smirnov. Event-free survival (EFS) was determined with the Kaplan–Meier and method, calculating time, status, cumulative survival, and standard error with a 95% confidence interval (CI) comparing autologous and allogeneic HSCT grafts. Equality of data distribution was estimated with the log-rank test. A two-sided p-value of 0.05 was considered statistically significant. For the analysis of non-parametric data comprising two datasets a Willcoxon-signed rank test was used, for the analysis of three or more datasets the Kruskal–Wallis test was performed.

3. Results

Twenty-seven patients were evaluated. Nineteen (70.4%) received an allogeneic HSCT, whereas eight (29.6%) received an autologous graft. General characteristics of patients included are shown in Table 1. Information about median time to transplantation, dose of CD34+ cells, neutrophil and platelet engraftment timing, as well as blood count on the day of bone marrow biopsy is displayed in Table 2.

By flow cytometry analysis the median values and interquartile ranges (IQ25 and IQ75) of the percentages of CD133/CD34 cell subpopulations in BM and PB samples from both allogeneic and autologous transplanted patients were determined. While analyzing the whole

Table 1
General characteristics and diagnoses of 27 hematopoietic stem cell transplant recipients, distributed in 19 allogeneic and 8 autologous patients.

Parameters	Allogeneic n: 19 (%)	Autologous n: 8 (%)	p
Gender			0.405
Female	9(47.4)	2(25)	
Male	10(52.6)	6(75)	
Age	24(3–65)	55(28–59)	0.013
Diagnosis			≤0.001
Leukemia	10(58.8)	0(0)	
Myeloma	0(0)	4(50)	
Myelodysplastic syndrome	5(29.4)	0(0)	
Lymphoma	2(11.8)	4(50)	