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Long-term immune recovery after CD34+ immunoselected and unselected peripheral blood progenitor cell transplantation: a case-control study

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ABSTRACT

Background and Objectives. **CD34+ stem cell selection induces extensive T-cell depletion as a consequence of *ex vivo* manipulation. The impact of T-cell depletion on long-term immunologic recovery after autologous CD34+ peripheral blood progenitor cell transplantation (CD34+ PBPCT) is not well characterized. We compared the long term immunologic recovery in two groups of patients submitted to CD34+ PBPCT or unselected autologous peripheral blood progenitor cell transplantation (uPBPCT).**

Design and Methods. **Eight patients in both groups were closely matched for diagnosis, age, disease status at transplantation and conditioning regimen and lymphocyte phenotype was prospectively evaluated during long-term post-transplantation follow-up.**

Results. **At a median of 18 months after transplantation, CD3+ lymphocyte subset remained below the normal range in both groups. CD19+ B lymphocytes subset after CD34+ PBPCT was within the normal range in both groups. CD4+ lymphocytes were depressed while the CD8+ lymphocyte subset was increased in group A and in the normal range in group B. As a result, inversion of CD4/CD8 ratio was documented in both groups. T-activated lymphocytes (CD3DR+) and natural killer (CD16/56+) cells were increased in both groups.**

Interpretation and Conclusions. **Long-term immune recovery appears to be unaffected by extensive *ex vivo* manipulation in this adult population when compared to recovery after unmanipulated PBPCT. CD34+ selection, although causes an extensive depletion of T lymphocytes in the graft does not represent a risk factor for delayed CD4+ recovery late after transplantation. Elevated numbers of NK cells and activated T-cells, which have antineoplastic activity, are maintained late after autologous CD34+ transplantation.**

Key words: immune recovery, immunoselected CD34+ PBPCT, unselected PBPCT, late effects, autologous transplantation

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Autologous transplantation using mobilized peripheral blood progenitor cells (PBPC) produces a more rapid hemopoietic recovery than autologous bone marrow transplantation (ABMT). Peripheral blood progenitor cell transplantation (PBPCT) also requires less supportive care and shorter hospitalization.[1,2] In addition to the above-mentioned advantages of PBPCT, this procedure may also induce a faster recovery of immune function than ABMT as a result of the large number of lymphocytes infused with the graft.[3,4] Since PBPCT was introduced into clinical practice, it has become clear that neoplastic contamination of the graft is not abrogated by using peripheral blood and several attempts have been made to reduce contamination by neoplastic cells. CD34+ cell selection reduces contamination from tumor cells not bearing the CD34 antigen on their surface but also induces a profound depletion of T lymphocytes in the graft. As a result of this procedure, immunologic reconstitution could be delayed and incomplete. We report here on the results on long-term immune recovery in patients submitted to autologous CD34+ PBPCT.

Design and Methods

Patients included in this study had to fulfill the following criteria: minimum follow-up after transplantation of 12 months, continuous complete remission without receiving additional chemotherapy, radiotherapy or other biological response modifiers. Eight patients submitted to CD34+ PBPCT (group A) were sorted and compared to 8 patients submitted to uPBPCT (group B). Patients were matched for diagnosis, age, disease status at PBPCT and conditioning regimen. Immunologic recovery was evaluated at 12 months and every 6-12 months during long-term follow-up after transplantation.

Group A

Eight immunoselected autologous CD34+ PBPC transplants (Ceprate SC, Cellpro, Bothell, WA, USA) were performed in 8 patients. Six patients were male and 2 patients were female with a median age of 45.5 years (range 22-62). Three patients were affected by multiple myeloma, 3 by non-Hodgkin's lym-

phoma, and 2 by Hodgkin's disease. Five patients were in complete remission and 3 patients were in partial remission at the time of transplantation. The conditioning regimen was BUMEL in 6 patients and BEAM in 2 patients. Median follow-up post-transplantation was 19.5 months (range 12-31). The median number of $CD3^+$ T-cells infused was 0.011×10^6/kg/bw (range 0.0014-0.02). $CD4^+$ T-cells were undetectable in all samples tested after immunoselection (<0.01% of analyzed cells). The purity of the $CD34^+$ cells' concentrate was 90.1%. The median value of $CD34^+$ cells was 7.95×10^6/kg/bw (range 1.7-16). The patients' characteristics are shown in Table 1.

Group B

Eight patients submitted to uPBSCT were chosen to match group A patients closely. Patients were comparable in terms of age, disease, disease status at transplantation and conditioning regimen. Median follow-up post-transplantation was 18 months (range 12-30). The median number of $CD3^+$ T cells infused was 37.65×10^6/kg/bw (range 3.85-62.9). The number of $CD4^+$ subset T-cells infused was 17.07×10^6/kg/bw (range 2.1-44.72). The median value of $CD34^+$ cells infused was 15.5×10^6/kg/bw (range 0.7-50.2). The patients' characteristics are shown in Table 2.

Lymphocyte phenotype

Samples were obtained annually during the post-transplantation follow-up. Short-term immunologic reconstitution has been reported elsewhere.[5] Briefly T-cell immune reconstitution during the first year was markedly depressed in patients receiving immunoselected $CD34^+$ progenitors as compared to patients transplanted with unfractionated PBPC. Double labeling experiments were performed on EDTA anticoagulated blood samples; aliquots of 100 mL were incubated for 30 minutes at 4°C with FITC or PE conjugated moAb: CD45, CD4, CD8, CD16, HLA-DR, CD56. Isotype-matched control antibodies were used as controls. Erythrocytes were lysed by adding 3 mL of NH_4Cl/EDTA for 10 minutes at room temperature; cells were then washed in PBS-EDTA and kept on ice until FACS analysis (FACScan, Becton Dickinson, USA). The expression of $CD45RA^+$ and $CD45RO^+$ isoforms on T-cells was not evaluated in this retrospective analysis.

Statistical evaluation

Comparison analysis was performed using a non-parametric test (Mann-Whitney test), defining the criterion for statistical significance as $p<0.05$.

Results

All patients transplanted with selected and unselected PBPC achieved rapid and stable hematopoietic engraftment.

In both groups we observed a persistent reduction of the CD4/CD8 ratio. Median value of the ratio was 0.3 in group A (range 0.2-0.7) and 0.6 in group B (range 0.3-1.2) (p=0.05) mainly due to a persistent reduction of $CD4^+$ T-lymphocytes in both groups with an increase and a relatively normal $CD8^+$ T-cell subset, respectively in group A and group B. The $CD8^+$ lymphocyte subset was increased in both groups (p=ns). No difference was observed in the number of $CD3^+$ T cells which was below the normal range in both groups. NK cells ($CD16/CD56^+$) and activated T-cells ($CD3DR^+$) were increased in both groups (p=ns). The $CD19^+$ B-cell subset was normal in group A and showed a slight increase in group B (p=ns). Data are shown in Table 3.

Table 1. $CD34^+$ characteristics of patients.

Dx	Age	Disease status at PBPCT	Conditioning regimen	$CD34^+$ $x10^6$/kg reinfused	$CD3^+$ $x10^6$/kg reinfused	$CD4^+$ $x10^6$/kg reinfused	Follow-up post-PBPCT mos.
HD	32	CR	BEAM	8.8	0.015	n.e.	13
HD	22	CR	BEAM	10	0.0017	n.e.	12
NHL	43	PR	BUMEL	3.8	0.018	n.e.	31
NHL	40	CR	BUMEL	1.7	0.0036	n.e.	15
NHL	48	PR	BUMEL	7.1	0.02	n.e.	24
MM	60	PR	BUMEL	16	0.0084	n.e.	24
MM	58	CR	BUMEL	10.76	0.016	n.e.	18
MM	62	PR	BUMEL	2.5	0.0014	n.e.	21
Med.	45.5			7.95	0.0011		19.5

Legend: Dx: disease; HD: Hodgkin's disease; NHL: non-Hodgkin's lymphoma; MM: multiple myeloma; CR: complete remission; PR: partial remission; n.e.: not evaluable; mos.: months; Med.: median.

Table 2. PBSC characteristics of patients.

Dx	Age	Disease status at PBPCT	Conditioning regimen	$CD34^+$ $x10^6$/kg reinfused	$CD3^+$ $x10^6$/kg reinfused	$CD4^+$ $x10^6$/kg reinfused	Follow-up post-PBPCT mos.
HD	26	CR	BEAM	18.7	15.84	4.15	25
HD	36	CR	BEAM	1.99	62.9	44.72	12
NHL	54	PR	BUMEL	32.8	3.85	2.1	12
NHL	27	CR	BUMEL	0.7	46.6	29.82	12
NHL	46	PR	BUMEL	50.2	7.58	4.67	12
MM	35	PR	BUMEL	6.4	44.65	19.3	29
MM	51	CR	BUMEL	15.5	30.66	17.25	30
MM	51	PR	BUMEL	n.e.	55.64	16.9	24
Med.	41			15.5	37.65	17.07	18

Legend: Dx: disease; HD: Hodgkin's disease; NHL: non-Hodgkin's lymphoma; MM: multiple myeloma; CR: complete remission; PR: partial remission; n.e.: not evaluable; mos.: months; Med.: median.

Discussion

Immune reconstitution after PBPCT has been extensively studied since the 1970s with the demonstration of long-standing post-transplantation cellular and humoral immunodeficiency.[6] The recovery of $CD3^+$ T-cells generally occurs within 6 months after trans-

Table 3. Immunological reconstitution.

Lymphocytes subset	Median value (range) A group	B group	normal value	p (t-test)
CD4×10⁹/L	0.269 (0.189-0.472)	0.399 (0.247-0.926)	0.670-0.950	0.126
CD8×10⁹/L	0.906 (0.388-1.294)	0.594 (0.208-2.205)	0.505-0.695	0.838
CD4/CD8	0.3 (0.2-0.7)	0.6 (0.3-1.2)	1.1-1.8	0.055
CD3×10⁹/L	1.129 (0.724-1.568)	1.007 (0.403-3.263)	1.185-1.540	0.811
CD19×10⁹/L	0.294 (0.113-0.742)	0.515 (0.131-0.767)	0.160-0.290	0.232
CD3/DR×10⁹/L	0.363 (0.132-0.678)	0.250 (0.091-0.882)	0.040-0.155	0.640
CD16/56×10⁹/L	0.195 (0.112-0.254)	0.221 (0.130-0.706)	0.070-0.190	0.144

plantation although a markedly decreased CD4/CD8 cell ratio remains for a longer time.[7-9] We analyzed long-term immune reconstitution in patients submitted to immunoselected autologous CD34⁺ PBPCT for hematologic malignancies. In fact, as a result of CD34⁺ selection, massive T cell depletion is usually carried out and a negligible amount of T-cells is normally present in the graft. It has been reported in fact that after CD34⁺ selection using either an immunomagnetic or an immunoadsorbtion technique, a 3-4 log T-cell depletion is achieved.[10] Thus it is conceivable that after reinfusion of autologous CD34⁺ stem cells, immune recovery would rely primarily on proliferation and differentiation from a multipotent stem cell. As a result, delayed and incomplete immune recovery is expected particularly in comparison with recovery after unselected PBPCT. In fact, it has been calculated that at least 20% of peripheral blood cells reinfused after conventional PBPCT are CD3⁺ lymphocytes contributing to a very fast immune recovery of this T-cell compartment. Our results show that a significant reduction in CD4/CD8 ratio occurs late after immunoselected CD34⁺ PBPCT. The reduction of CD4/CD8, although more pronounced than in a group of patients submitted to uPBPCT, did not reach statistical significance probably because of the small sample size. CD4/CD8 was due to the reduction of CD4⁺ T-cells with a relatively normal CD8⁺ T-cell count and has already been demonstrated. The extrathymic origin of these cells[11] accounts for this rapid recovery, at least in adult patients. In contrast, CD4⁺ subset recovery, which is thymus-dependent, appears to be profoundly impaired after PBPCT as expected in this adult population.[12] An unexpected finding was that a more pronounced increase in CD8⁺ T cells was observed after CD34⁺ PBPCT than after uPBPCT. Similar results have been recently reported after transplantation of FACS sorted CD34⁺ hematopoietic progenitors.[13] In this setting, in which an even more pronounced T-cell depletion is obtained, short-term immune reconstitution appears to be delayed and a decreased diversity of the T-cell repertoire has been demonstrated. CD3⁺DR⁺ T-cells were increased in both groups while NK (CD16/56⁺) cells were in the normal range by 1 year post-transplant.[13] A persistent increase of B-cells (CD19⁺) in the two groups was also noted, thus B-cell reconstitution after CD34⁺ immunoselected PBPCT seems to be rapidly restored from primitive hemopoietic precursors. These data compare favorably to those observed by Vescio *et al*. in a randomized trial for multiple myeloma using the same technique for CD34⁺ selection.[14]

In conclusion, the long-term immune recovery after CD34⁺ selected PBPCT appeared not to be different to that after uPBCT with the exception of a more pronounced reduction of the CD4/CD8 ratio. These observations contribute to the documentation of safety after manipulation of autologous stem cells for hematologic malignancies and may also be useful in designing trials for non-neoplastic disorders, particularly autoimmune diseases.

Contributions and Acknowledgments

LL was the principal clinician involved and responsible for the study design, SS was responsible for the interpretation of data and supervision, FS and NP were responsible for data handling and statistical analysis, EOLB PC and PS were responsible for data handling, immunoselection procedures and long-term follow-up, CR and SR were responsible for all immunological data, and GL revised the manuscript and gave final approval. The order in which the authors' names appear reflects their contributions to the study.

Disclosures

Conflict of interest: none.

Redundant publications: <50%. Although some results have already published in Haematologica this work provides further evidence of comparable long-term immune recovery in patients submitted to autologous CD34⁺ PBPCT⁺. The number of patients previously reported in Haematologica has almost doubled. The patients population has been matched to a group of unselected PBPCT in order to avoid bias generated by the heterogeneity of patients analyzed.

Manuscript processing

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