Autologous peripheral blood stem cells: collection and processing

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The rapid development in the area of collecting and processing autologous peripheral blood stem cells (PBSC) is reflected by the escalating number of patients treated with PBSC, and by the increasing amount of literature on the subject. Clinical experience suggests that among the variables with a negative influence on mobilization of PBSC, the most important may be the amount of previous stem cell toxic chemotherapy. In selecting patients suitable for autologous PBSC support, the requirement of an adequate anti-tumor therapy has to be weighed against the risk of chemotherapy related stem cell toxicity which will result in inability to collect a sufficient amount of PBSC. The general consensus is that a sufficient PBSC-autograft should contain 2-5 × 10⁶ CD34⁺ / kg body weight, but attempts to provide a recommended optimal or threshold level are hampered by the lack of standardized methods for CD34⁺ cell enumeration. In addition, the time to haematological recovery depends both on the dose of infused CD34⁺ cells and also on the amount of previous chemotherapy, which affects both the quality of the graft and the supportive microenvironment of the host. The quality of the autograft may also be contaminated by malignant cells, even if the biological significance of tumor cell detection in the PBSC graft has not yet been established. Recent development of methods for in vitro purging and selection of CD34⁺ cells for clinical use have provided the means to avoid or reduce reinfusion of malignant cells. Future directions of clinical research include the ability to define and enumerate the proportion of stem cells versus committed progenitor cells among the CD34⁺ cells in a PBSC collection, which will be important to ensure rapid engraftment as well as long term haematopoiesis.

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INTRODUCTION

The use of autologous peripheral blood stem cells (PBSC) for haematopoietic reconstitution after high-dose chemotherapy has become an alternative to allogeneic or autologous bone marrow (BM) transplantation. Treatment protocols utilizing mobilized PBSC after ablative high-dose chemotherapy, with or without total body irradiation, or as haematopoietic support after repeated courses of high-dose chemotherapy, are being used for both haematological malignancies and solid tumors [1-6].

There are several potential advantages with autologous PBSC over BM: (1) collection of PBSC by apheresis is an out-patient procedure not requiring general anaesthesia and hospitalization; (2) PBSC can also be harvested from patients with previous pelvic irradiation and from patients with BM engaging malignancies; and (3), possibly the most important, there is an accelerated haematopoietic recovery of all lineages as compared to BM-transplantation [1,7-8].

An adequate number of PBSC can be collected from the blood by timed leukapheresis procedures during the rebound period after chemotherapy and haematopoietic growth factor administration (granulocyte- or granulocyte-macrophage colony stimulating factor, G- or GM-CSF). In particular
MOBILIZATION OF PBSC

In order to collect a sufficient number of progenitor cells by a limited number of apheresis procedures for an autologous reinfusion after myeloablative therapy, mobilization with growth factors should be induced and apheresis performed during the mobilization period. Optimal mobilization is a brief event (2-4 days) that can be predicted and followed for each patient by monitoring monocyte recovery and the level of CD34+ cells in the blood during leukocyte recovery after chemotherapy [16]. Although CD34 is expressed by both stem cells and lineage committed progenitor cells, a positive correlation between the number of CD34+ cells and the number of functional progenitor cells (as determined by their colony growth) suggests that CD34 can be used as a reliable marker for quantity and quality of PBSC [12-16].

Several parameters may affect the level of mobilization, i.e. individual variation, BM-involvement of the malignancy, growth factor dose and route of administration, but the most important may still be the amount and degree of stem cell toxicity from previous chemotherapy. Thus, it was recently shown for patients with lymphoma (HD and high-grade NHL) that the best predictive parameter for successful collections of PBSC was ≤1 course of the stem cell toxic regimen Dexamethasone, BCNU and melphalan, sequential high-dose carboplatin has also been reported to affect the level of circulating stem cells [17]. In addition, the immuno-modulating cytokine Interleukin-2, used either alone or in combination with chemotherapy, has a known inhibitory effect on haematopoiesis [18] and should be omitted in time (according to our experience at least 4 weeks) before mobilization of PBSC. Because of these adverse effects on PBSC mobilization, ≥6 months prior therapy is often used as an exclusion criteria in clinical trials with high-dose chemotherapy with PBSC support for multiple myeloma. The strategy is to avoid stem cell toxic chemotherapy in order to allow mobilization of PBSC, but also to provide adequate therapy for tumor reduction and an optimal in vivo purging to reduce the risk of tumor cell contamination in the autologous graft.

Most likely, both the mobilization phase and the recovery phase after PBSC reinfusion, is affected by the amount of previous chemotherapy [5]. Whether this is a sign of the poor quality of the harvested stem- and progenitor cells, a selection against the progenitor pool, or reflects a damaged BM micro-environment unable to support fully the newly repopulating PBSC, remains to be understood.

In addition to selecting patients without prior stem cell toxic chemotherapy for PBSC-harvest, the time of harvest after chemotherapy and growth factor mobilization also contributes to the outcome of the PBSC collection. Apheresis should be performed at a time when the numbers of circulating progenitors and stem cells are high enough to assure a beneficial yield in the resulting PBSC harvest. Most centres perform the apheresis according to their own experiences and logistics, with or without a daily monitoring period to detect CD34+ cells. Thus, collections may start at a fixed day, at the time when WBC counts rise > 1 × 10^9/l [13], >5 × 10^9/l [12] or above 10 × 10^9/l [5]. From our experience in monitoring a heterogeneous group of patients, we concluded that the time of optimal mobilization (of both CD34+ cells and GM-CFU) varied between the patients from day 8 to day 24 after the last day of chemotherapy, with a median of 12 days [16]. In these patients, the WBC count was not a reliable predictor of optimal mobilization since WBC counts ranged from 3.8 to 49 × 10^9/l (median 14.4 × 10^9/l) the