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Novel cell population data from a haematology analyzer can predict timing and efficiency of stem cell transplantation

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ABSTRACT

Autologous hematopoietic stem cell transplantation (AHSCT) is a necessary component for many oncohematological diseases treatment. For a successful result of AHSCT a sufficient quantity of hematopoietic stem cells (HSCs) is needed. It has been proposed that morphological changes of myeloid cells could reflect the processes of bone marrow stimulation and may provide useful information to predict the stimulation efficiency and expected outcome of $CD34^+$ stem cells. The Beckman Coulter Cellular Analysis System DxH800 performs Flow Cytometric Digital Morphology analysis of leukocytes. All leukocyte cellular measurements can be reported as numerical values called Cell Population Data (CPD), which are able to detect morphological changes in the cell size and distribution of neutrophils. Our findings suggest that the changes in neutrophil CPD were detectable 2–4 days before the observed increase in $CD34^+$ count in the peripheral blood and can potentially improve the management of patients. There was also a good correlation between MN-V-NE and ImmNeIndex with the $CD34^+$ count suggesting they can be used as a surrogate for the $CD34^+$ count ($r = 0.67$ and 0.65 $p < 0.005$ respectively).

1. Introduction

The first transplantation of human hematopoietic stem cells (HSCs) to human recipient was performed nearly 45 years ago. In 2010, in Europe more than 30,000 HSC transplantations were done, which demonstrates growing attention to this procedure as a powerful treatment modality for a variety of diseases [1]. In treating hematologic and non-hematologic malignancies, autologous HSC transplantation are frequently used to rescue hemopoiesis after a high dose-chemotherapy. Treatment of congenital and acquired marrow failure with allogeneic HSC transplantation has been widely used.

Historically, HSC were isolated from the bone marrow, which was harvested by repeated aspirations from the ileac crest. Today, mobilized HSC from peripheral blood (PB-HSC) are used for transplantation. Autologous transplantation of PB-HSC is widely used to treat diseases according to published guidelines [2]. For the purposes of transplantation, PB-HSC are collected as mononuclear cells by apheresis. The number of viable HSC per kilogram of recipient body weight is a critical factor determining the success of PB HSC transplantation. Transplanting of insufficient number of HSC normally results in lower efficiency of replacement of the hematopoietic component in patients with oncohematological diseases and can lead to an increased risk of disease relapse.

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Main sources of HSC for clinical transplantations are:

(1) Unmodified biological sources:
   (i) Peripheral blood.
   (ii) Bone marrow (BM).
   (iii)
   Cord blood (CB).
(2) Modified biological sources: blood cells, purified from cells without the abilities of stem cells precursors (erythrocytes, granulocytes). Normally, clinical grade PB-HSC are derived by apheresis [3].

In healthy non-stimulated donors, HSC constitute a minor fraction in the PB (0.01–0.1% of all cells), while the BM contains higher amounts (1–3% of all cells). Mobilization of HSC from the BM to PB can be achieved by administering granulocyte colony stimulating factor (G-CSF) to a donor, which usually increases the levels of HSC in PB up to 1.5–2%. G-CSF-mediated mobilization of HSC occurs in two stages [4]. The first stage involves differentiation of granulocyte lineage progenitor cells into more mature forms. During the second stage alteration of proteolytic microenvironment in the bone marrow occurs, with increase in enzymes such as neutrophil elastaze, catepsin G, proteinase 3 and others [5–11]. At this stage HSC activation sites in the bone marrow microenvironment are suppressed, predominantly through the increase of the CXCR4-SDF-1 chemokine concentration. CXCR4-SDF1 acts to retain HSC in the BM and prevents their release into the PB. To overcome this retention, a specific CXCR4-SDF-1 inhibitor (Plerixifor) can be used together with G-CSF to stimulate HSC mobilization [12].

G-CSF regulates the amount of neutrophils produced and the exit of functional neutrophils from BM to PB [13–17]. Mobilized neutrophils were shown to be highly granulated and biologically active, and there is a direct correlation to their number in PB correlates directly with the dose of administered G-CSF. Based on cell-mobilizing effect of this cytokine, it was presumed that changes in neutrophils populations's pattern such as the appearance of immature forms following G-CSF administration could predict the best time point to perform apheresis. These changes in neutrophil patterns might be used as an indirect indicator of an increase of PB HSC count. The evaluation of the mobilization success is performed on the basis of the HSC sufficiency collected in the course of the apheresis performed after one full course of the mobilization [18,19].

The immunophenotype of pluripotent HSC has been well characterized and includes membrane markers CD34, CD133, c-kit (CD117) and absence of the CD38 and specific markers of the lineage-committed cells: GlyA, CD2, CD3, CD4, CD8, CD14, CD15, CD16, CD19, CD20, CD56, and CD66b (Lin-). Because of convenience, $CD34^+$ counting has become the gold standard for measuring HSC numbers and evaluating restoration of haematopoiesis following BM transplantation. Studies of HSC mobilization efficiency evaluated by $CD34^+$ counts allowed to categorize transplantation patients into three groups [20]. In non-mobilizable patients, repeated apheresis fails to produce more than 2×10^6 $CD34^+$ HSC per kg. In patients of the other group, only $2–5 \times 10^6$ $CD34^+$ HSC/kg are collected totally after repeated apheresis, while in easy-to-mobilize patients more than 5×10^6 $CD34^+$ HSC/kg can be collected by three to five repeats of apheresis procedure.

To measure HSC numbers, $CD34^+$ cell counts can be determined by counting total leukocytes with a hematology analyzer followed by counting $CD34^+$ cells by flow cytometry. Alternatively, both total leukocyte and $CD34^+$ cell counting can be performed by using a single platform: a flow cytometer with volumetric capabilities, or a regular flow cytometer and a kit of graduated fluorescent beads.

The ISHAGE [21] protocol is based on obtaining the maximum available information from four parameters: forward and side light scattering and the intensity of CD34 and CD45 fluorescence. The sequential estimation of these parameters allows accurate counting of HSC in different apheresis products. By using ISHAGE, hematopoietic progenitor cells are detected based on their low granularity and low level of CD45 expression, while the cells that bind CD34 antibodies non-specifically are efficiently excluded. An example of a commercially available single platform kit is the Stem-KIT™ from Beckman Coulter [Brea, California, USA], which includes two-color combination antibody reagents, CD45FITC/CD34PE, CD45FITC/isotypic control-FITC, the viability dye 7-AAD, ammonium chloride as the lysing solution, along with Stem-Count™ beads (Beckman-Coulter). This kit allows direct evaluation of the absolute amount of $CD34^+$ cells. The analysis logic complies with one-platform guidelines published by ISHAGE [21]. Additionally, Stem-KIT™ includes Stem-ONE™ software, which automatically calculates $CD34^+$ cell count. Some of the challenges of obtaining correct $CD34^+$ counts include the availability of a flow cytometry laboratory to test patient samples and apheresis products round the clock. Furthermore, $CD34^+$ counting is relatively expensive, making the search for alternative, less costly and easier to obtain markers highly actual for HSC transplantation management.

Therefore we sought to investigate if there were more rapid and less costly parameters available on a haematology analyzer, the Beckman Coulter DxH800, which could predict the HSC count ($CD34^+$) in patient samples and apheresis products. Study design included investigation of novel Neutrophil Cell Population Data generated with Beckman Coulter DxH 800 haematology analyzer against the reference ISHAGE $CD34^+$ counting method in patients with various haematological malignancies undergoing autologous HSC transplantation.

2. Materials and methods

2.1. Patient cohort

Our dataset contained thirteen patients with multiple myeloma (MM, $n = 10$) and Hodgkin's disease (HD, $n = 3$), who were included in the study after giving an informed consent. The cohort consisted of ten females and three males with age ranging from 21 to 59. The patients underwent different mobilization regimes (Table 1). Totally, 26 apheresis products from 13 patients' samples and 126 PB