Abstract

Immunophenotyping of CD34+ cells by flow cytometry method

CD34+ hematopoietic stem cells (HSC) are undifferentiated cells with the ability to differentiate, proliferate and self-renew. The discovery of specific surface markers unique to human HSC enabled the investigation of their development from HSC to mature hematopoietic cells. Today, transplantation of HSC is a routine method in the treatment of patients with hematological, oncological and immunological diseases. HSC are normally present in peripheral blood in an extremely small number ($<10x10^6$/L), thus they belong to the so-called rare events considering that they are primarily located in the bone marrow. Their mobilization, i.e. increase in peripheral blood, is possible with the use of high doses of radiation and/or chemotherapy, granulocyte colony growth factor (G-CSF) and chemokine inhibitors (plerixafor). Transplantation of HSC means the procedure of taking healthy HSC from peripheral blood, umbilical blood or bone marrow, freezing them and re-infusing the patient. There are two types of HSC transplantation: autologous, where the donor of HSC is the patient himself, and allogeneic, where the recipient and the donor of HSC are different people. The advantage of autologous transplantation of HSC is the absence of the risk of graft-versus-host disease (GvHD), and the disadvantage is the possible presence of tumor cells. During allogeneic transplantation of HSC, the frequency of graft-versus-recipient reaction is increased, but the advantage is the graft-versus-tumor effect (GvT).

Sutherland et al. have developed a sensitive and precise protocol for immunophenotyping by the flow cytometry method that uses four parameters: cell size (light scattered at a small angle of 0.5°-10°, FSC - forward scatter) and granularity (light scattered at a 90° - SSC, side scatter) and the intensity of expression of CD34 and CD45 markers. Unique to this protocol is the use of successive gating, along with analysis of a population of interest that is suitable for different sources of HSC. This protocol forms the basis of clinical guidelines for determining the number of HSC, and in 1996 the ISHAGE protocol (*International Society of Hematotherapy and Graft Engineering*) was published for the simple determination of the relative proportion of HSC (*DUAL* platform) where the absolute number of leukocytes determined on a hematology counter is used to determine HSC for immunophenotyping by the flow cytometry method. Not long after, a modified ISHAGE protocol was presented that uses exclusively Immunophenotyping by the flow cytometry method by adding a known concentration of fluorescent particles, which enables the simultaneous determination of the absolute and relative number of HSC as well as the absolute number of leukocytes in the sample (*SINGLE* platform). The differentiation of leukocyte populations is currently more accurately displayed in the 2-parameter histogram of the combination of CD45 vs SSC. Cells with weak CD45 expression and low SSC are, as a rule, HSC and basophilic granulocytes. Lymphocytes have the same level of SSC, but with a strong CD45 expression. Monocytes have higher SSC and approximately the same intensity of CD45 expression. Granulocytes have weak to moderate expression of CD45, and medium to high SSC in size. CD45 is a pan-leukocyte marker, and selecting the CD45+/SSC histogram excludes platelets, insufficiently lysed erythrocytes and aggregates from the analysis. The next step is to select events that are in addition to CD45+ and CD34+ from the CD45+ population (leukocytes). After that, events that are CD34+ but have a lower level of expression of the CD45 marker (CD45dim) compared to the level of expression of the CD45 marker on lymphocytes are selected.

Further successive gating of the population of interest (HSC) is based on size (FSC) and granularity (SSC) in such a way as to select events that are CD34+CD45dim but that correspond in size to the location of lymphocytes. In order to obtain the absolute number of HSC as well as the absolute number of leukocytes, fluorescent beads of precisely known concentration in a precisely defined volume are added to the sample. Collection of HSC from peripheral blood (leukapheresis procedure) begins when the number of HSC ≥ 10 $x10^6$/L, and the number of leukocytes in the peripheral blood is ≥ 1.0$x10^9$/L or higher. With a good donor response, up to four leukapheresis procedures are required to collect an adequate number of HSC (2-5 x 10^6 per kg recipient body weight.

Key words: CD34+ hematopoietic stem cells, ISHAGE protocol, flow cytometry

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