A RAPID FLOW CYTOMETRY METHOD TO RAPIDLY DETERMINE THE STEM CELL CLONAL POTENCY IN FRESH APHERESIS UNITS

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The clonal potency (CP) is the potential capacity of stem cells (SC) to differentiate into mature cell types. The CP is usually measured by colony-forming unit (CFU) methods, which evaluates the number of Granulocyte–Monocyte progenitor colony (CFU-GM) and Erythroid burst-forming units (BFU-E) in culture. However, these assays are poorly standardized and require 14 days to obtain the result. To rapidly determine the SC-CP, a fast flow cytometric method based on the measurement of intracellular phosphorylated STAT5 (pSTAT5) in CD34+cells in response to IL-3 stimulation was developed. Aim of this study was the comparison of the pSTAT5 assay with the CFU method in ten units of fresh SC collections. Briefly, 25 µl of stem cell apheresis suspension was diluted in 85 µl of Dulbecco's Medium. For each sample two wells were used, one as the unstimulated control, and the other one as the stimulated sample by adding 100 ng/mL IL-3. After an incubation the cells were fixed and permeabilized with 70% of methanol and stained with anti-CD45-FITC/CD34-PE mix and anti-STAT5(pY694) Alexa Fluor 647 overnight at 4°C. The results were expressed as the percentage of pSTAT5+CD34+CD45+ (p STAT5+%) cells in the IL-3-stimulated well. We obtained respectively a median of 46,7% of pSTAT5+%cells, 56,5 CFU-GMx104Kg and 42,2 BFU-ex104Kg. We didn't find significantly differences in CP of SC evaluated by both methods in patients with multiple myeloma treated with DaraVTD versus VTD. We didn't find significant differences in 4 patients treated to plerixafor vs untreated patients. We didn't find differences of %pSTAT5+ cells in patients aged <55 years (42%) vs patients aged >55 years (47.7%). Our preliminary results indicate that the pSTAT5 flow cytometric assay is complementary to the CFU method. This method is faster and more standardized than the CFU method. Further studies, including more cases are necessary to confirm our data.