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The Use of CD45/SSC Dot Plots in the Classification of Acute Leukemias

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The anti-CD45 antibody is the most used reagent for immunophotyping acute leukemias. The analysis of CD45/SSC dot plot is mandatory when analyzing leukemic cells because this graph allows cytometristes to isolate blast cells from other hematopoietic cell types.

CD45 also called "Leukocytes Common Antigen" is expressed on mature and immature hematopoietic cells with the exclusion of platelets, erythrocytes, elements belonging to the erythroid compartment, and highly immature hematopoietic precursors. It is well known that lymphocytes are brightly stained with CD45 and gives a weak SSC signal, monocytes got less CD45 on their membrane than lymphocytes with a slightly higher SSC signal while granulocytes are weakly stained with CD45 and gives a very high SSC compared to the other nucleated cells.

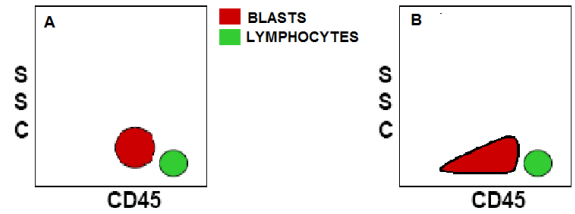

Figure 1: Schematic representation of Myeloblasts (A) and Lymphoblasts (B) compared to lymphocytes in a CD45/SSC dot plot according to Haycocks [1].

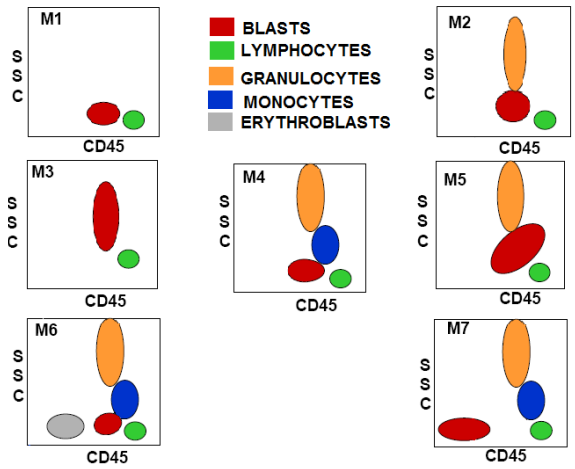

Figure 2: Correlation between different AML FAB subtypes and CD45/SSC dot plot patterns according to Saidi et al. [2].

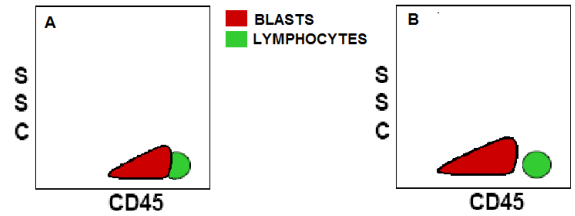

Figure 3: Pattern of CD45 expression on blasts (red) in Acute Lymphoblastic Leukemia (ALL) according to Ortolani [3]. On T lymphoblastic leukemia (T-ALL) blasts (A) CD45 can be expressed at a somewhat higher intensity than on B lymphoblastic leukemia (B-ALL) blasts (B), and can partially overlap the cluster of residual normal lymphocytes (green).

On a CD45/SSC bi-dimensional plot blast cells are located in the so-called "Bermude Area"; in the middle of the other described cell types. According to Haycocks, CD45/SSC cytogram allowed the flow cytometristes to classify immature leukemic cells into lymphoblasts and myeloblasts in 94, 1% of cases. In their paper the authors described the blasts of AML as a localized round/oval cluster with slightly higher SSC signal than lymphocytes (Figure 1) while the blasts of ALL as clusters distributed longitudinally along the CD45 axis with SSC signal similar to lymphocytes (Figure 2). Monoblastic population gives a very heterogeneous pattern which overlaps with the monocytic area [1].

Since different nucleated cells in a lysed whole blood and bone marrow can be quantified in a CD45/SSC plot, it is possible to make a correlation between the cytometric pattern and the FAB subtypes in AML. Figure 3 shows typical CD45/SSC patterns for in each AML FAB subtype according to Saidi et al. [2], but it should not be forgotten that on the promyelocytes of AML-M3v, CD45 is expressed at a higher intensity than on the promyelocytes of the classic form. In contrast, it is also possible that the blasts of sporadic cases of AML do not express the antigen; in such cases the immunological gate must be modified depending on the blast cell phenotype [3].

On T-lymphoblasts, CD45 is usually expressed at a lower intensity than on normal T lymphocytes, but at a somewhat higher intensity than on the cells of neoplastic diseases of B cell precursors. Consequently, the cluster of T leukemic lymphoblasts can partially overlap with the cluster of residual normal lymphocytes [3].

On the blasts of B-ALL, the intensity of CD45 expression is lower than on mature lymphocytes, and similar to the normal B precursors. But the intensity of CD45 on lymphoblasts is more heterogynous than on myeloblast that is why they are spread along the CD45 axis. However some special features of CD45/SSC in B-ALL must be kept in mind such as: 1) In cases with hyperdiploidy or with translocation t (12;21), CD45 can be missing or expressed at an intensity similar to erythroblasts. 2) In some cases with translocation t (4;11), the intensity of CD45 expression can be similar to mature lymphocytes. 3) Finally,in some cases with translocation t(9;22), a high expression of CD45 together with a high expression of CD19, CD22, CD34 and HLA-DR correlates with the presence of trisomy 8 [3].

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