Multicenter validation of a reproducible flow cytometric score for the diagnosis of low-grade myelodysplastic syndromes: results of a European LeukemiaNET study

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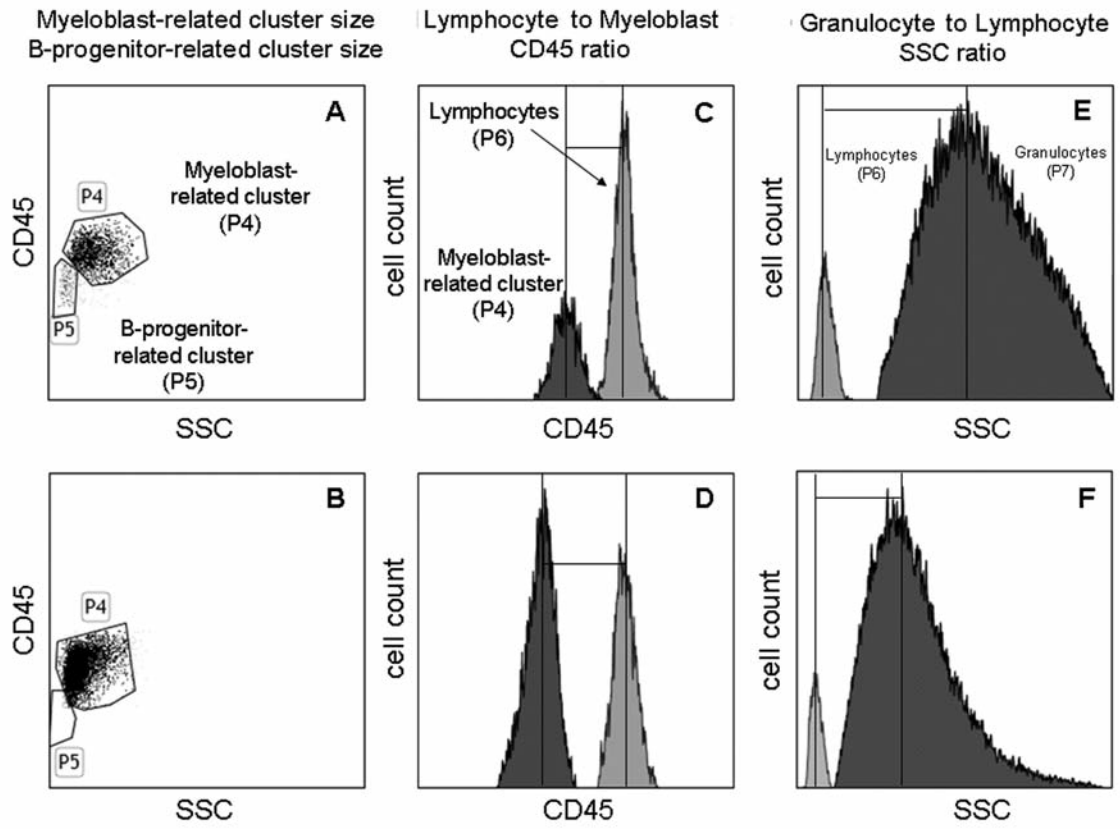
Online Supplementary Appendix

The reliability of the adopted gating strategy was verified in 30 patients enrolled in the study.

A significant correlation was found between the amount of "all nucleated cells" defined on the FSC-*versus*-SSC plot and that defined on both CD3/CD41b staining (in which "all nucleated cells" were defined as all cells with a size equal to or greater than that of lymphocytes, which are much larger than platelets) and a CD45/SSC plot (Spearman's rank correlation r=0.82, $P<0.001$, and r=0.87, $P<0.001$, respectively).

A comparison between different methods for detecting $CD34^+$ B-progenitors and $CD34^+$ myeloblasts was also performed. A strong correlation was found between the percentage of $CD34^+$ B-cell progenitors detected on the basis of SSC and CD45 characteristics (B progenitor-related cluster size) and both staining with CD34/CD10 and CD34/CD19 (r=0.87 and r=0.89, respectively, $P<0.001$). Compared with the method using the $CD34^+CD10^+$ phenotype, the method using the $CD34^+CD19^+$ phenotype detected fewer B-cell progenitors because some of these cells lacked CD19 expression, while the method using SSC and CD45 characteristics detected more B-cell progenitors.

A strong correlation was also found between the percentage of $CD34^+$ myeloblasts detected on the basis of SSC and CD45 characteristics (myeloblast-related cluster size) and staining with CD34/CD13/CD33 (r=.84, $P<0.001$). Compared with the method using the CD45 and SSC characteristics, the method using the $CD34^+CD13^+CD33^+$ phenotype detected fewer myeloblasts progenitors because some of these cells lacked CD13 and/or $CD33^+$ expression.



Online Supplementary Figure S1. Detection of marrow dysplasia by analysis of four cardinal parameters from a single sample of bone marrow cells stained with CD34 and CD45 antibodies. The gating strategy is shown in Figure 1. (A, C, E) Bone marrow from a representative subject with non-clonal cytopenia; (B, D, F) bone marrow from a representative MDS patient, showing an increased myeloblast-related cluster size (B), a decreased B progenitor-related cluster size (B), an increased lymphocyte to myeloblast CD45 ratio (D) and a reduced granulocyte to lymphocyte SSC ratio (F).