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Flow cytometric method for enumeration and classification of reactive immature granulocyte populations

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We developed a flow cytometric method for the enumeration and classification of nonmalignant immature granulocytes (IG). In this study, IG are defined as most immature (IG stage 1: promyelocytes and myelocytes) and as more mature (IG stage 2: metamyelocytes). Blood specimens from 46 patients with documented infectious or inflammatory disease and known presence of IG (by routine manual microscopy) were analyzed. For a reference manual differential count, we used a 400 white blood cell (WBC) differential and separated granulocytes into promyelocytes and myelocytes combined, metamyelocytes, and included band cells in the mature, segmented neutrophil population. The flow cytometric method is based on three-color staining of whole, anticoagulated blood with CD45-PerCP, CD16-FITC, and CD11b-PE-labeled monoclonal antibodies and a three-step gating procedure. The flow cytometric results were confirmed by cell sorting and microscopic evaluation of the sorted cells. A total of 10,000 events, excluding debris, were recorded per specimen and IG stage 1 (CD16-/CD11b-), IG stage 2 (CD16-/CD11b+), and mature neutrophils (CD16+/CD11b+) were categorized. Regression and correlation between flow cytometric IG and the manual differential showed $y = 1.34x + 0.95$, $r(2) = 0.86$ for IG stages 1 and 2 combined versus promyelocytes, myelocytes, and metamyelocytes. For IG stage 1 versus microscopic counts of promyelocytes and myelocytes, the results were $y = 1.53x + 1.24$, $r(2) = 0.76$; for IG stage 2 versus manual metamyelocyte count, $y = 0.77x + 0.21$, $r(2) = 0.58$. Reproducibility of the flow cytometric method showed a coefficient of variation (CV) of 6.8% for all IG combined compared with a CV of 50.2% for manual differential IG count (based on a routine 100 WBC count). Samples were found stable at least 12 h at 25 degrees C and at least 48 h at 4 degrees C for flow cytometry. After staining and lysing, the sample was stable for at least 120 min at room temperature. We analyzed samples from patients with myelodysplastic and myeloproliferative disease separately. We found that CD16- mature neutrophils falsely elevated the flow cytometric IG count. Similar results were obtained in blood from patients treated with granulocyte-colony stimulating factor (G-CSF). Although this restricts the use of the method somewhat, we believe that this flow cytometric method is useful for enumerating reactive IG, as well as for evaluating automated methods for IG identification by hematology analyzers