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Scientific Letters

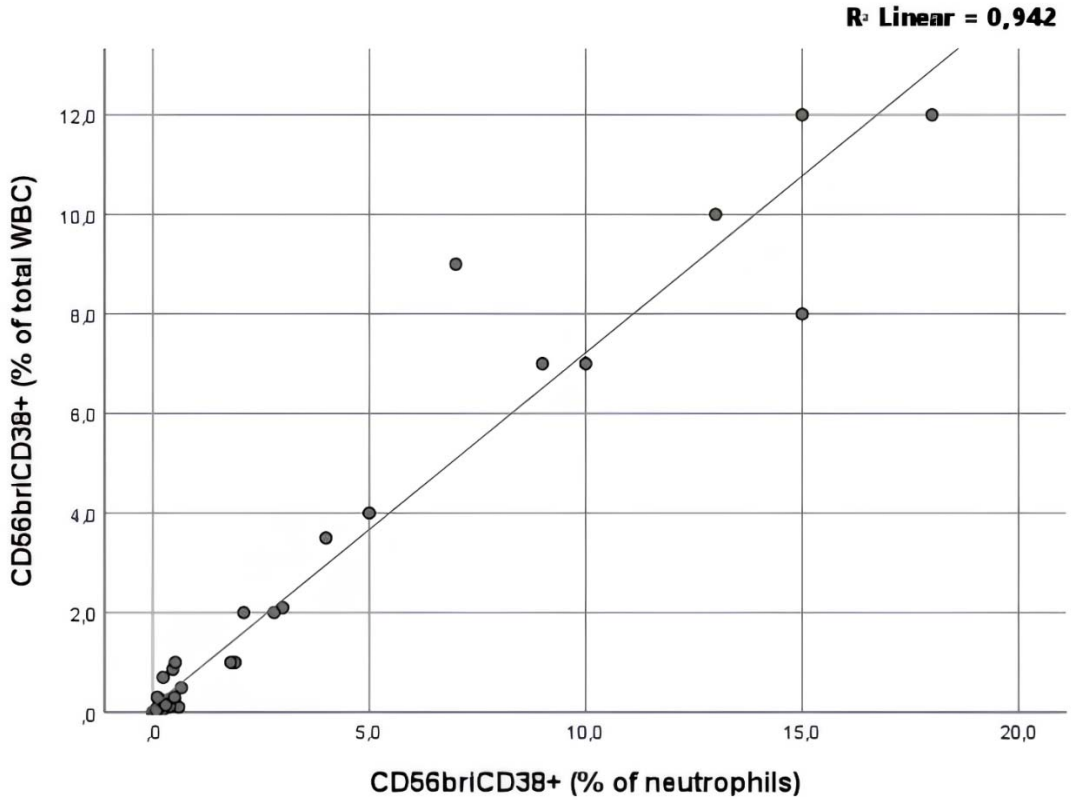
$CD56^{+}/CD38^{+}$ Neutrophils: Rapid and Specific Flow Cytometric Signature for Chronic Myeloid Leukemia

Supplementary Data

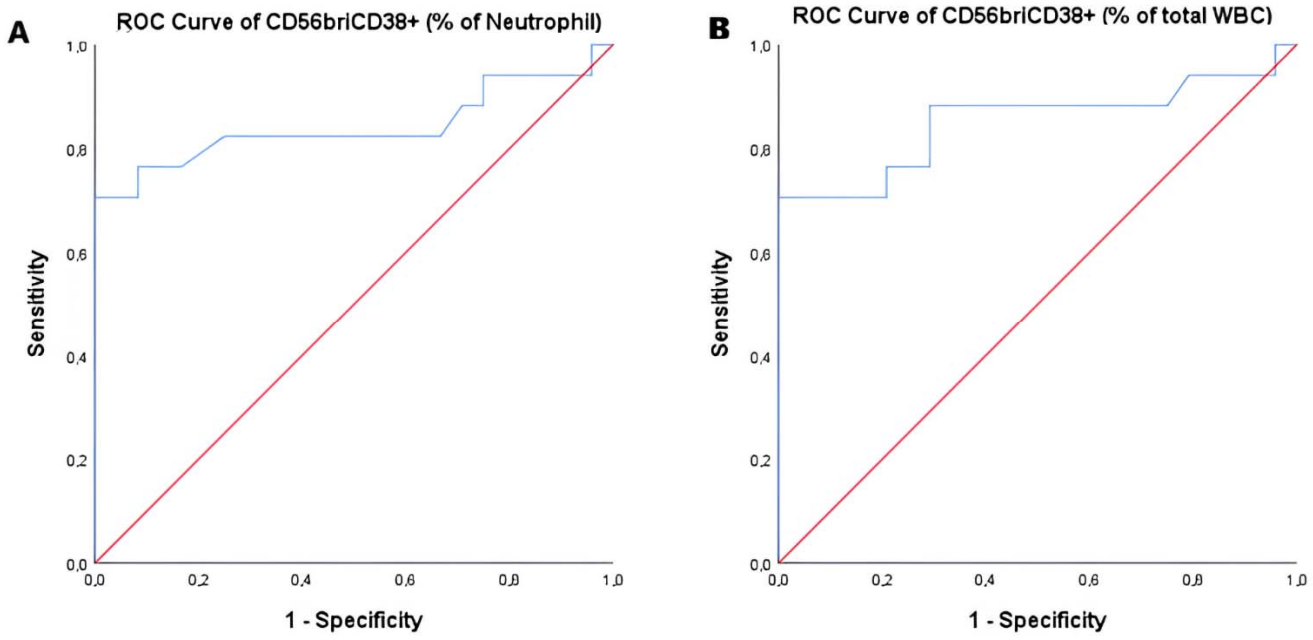
Flow Cytometry Supplementary Method

Peripheral blood (PB) samples were stained within 24 hours of collection, before any treatment. Total leukocytes were incubated with an appropriate volume of monoclonal antibodies (mAbs) directed against CD13/CD33/CD11b/CD15/CD10/CD16/CD56/CD38/CD34/CD26/CD45/CD117/HLA-DR (Società Italiana Chimici, SIC, Life Sciences, Rome, Italy; Beckman Coulter, Brea, CA). Data from standardized 12-color staining combinations were acquired on FACSCanto II or BD FACS Lyric flow cytometers (Becton Dickinson) and analyzed using PAINT-A-GATE and FACSDIVA software (Becton Dickinson). Instrument performance on the BD FACS Lyric™ was monitored daily using BD™ Cytometer Setup and Tracking (CS&T) beads to maintain stable photomultiplier tube voltages and consistent fluorescence sensitivity. For each sample, at least 50.000 $CD45^{+}$ events were acquired to ensure a high signal-to-noise ratio and precise quantification of rare populations. As mentioned in the manuscript, a comprehensive, sequential MFC gating strategy was employed to ensure accurate neutrophil identification. Within the neutrophil population, the subset of $CD56^{bri}CD38^{+}$ neutrophils was identified by CD38 positivity and the intensity of CD56 expression. In particular, identification of $CD56^{bri}$ neutrophils was based on CD56 Mean Fluorescence Intensity (MFI), using internal biological references within the same sample as comparison. Mature, non-aberrant neutrophils served as the negative control, and NK cells ($CD45^{+}/SSC^{low}/CD56^{+}$) provided the positive reference for CD56 "bright" staining intensity. Specifically, neutrophils were classified as $CD56^{bri}$ when their CD56 MFI was at least 1 log decade higher than that of the negative neutrophil population and similar to that of the NK-cell subset. Finally, all processed data were independently cross-validated by two expert flow cytometrists to mitigate inter-operator variability and ensure the scalability of this diagnostic tool in different clinical settings.

Supplementary Figures



Supplementary Figure S1. Scatter plot showing the relationship between the proportion of $CD56^{bri}CD38^{+}$ neutrophils expressed as a percentage of total $CD45^{+}$ WBCs and as a percentage of neutrophils. Each dot represents an individual sample. A strong positive linear correlation was observed (R^2=0.942).



Supplementary Figure S2. Receiver operating characteristic (ROC) curves showing the diagnostic performance of $CD56^{bri}CD38^{+}$ neutrophils for CML identification. **A)** ROC curve for $CD56^{bri}CD38^{+}$ neutrophils expressed as a percentage of total neutrophils. **B)** ROC curve for $CD56^{bri}CD38^{+}$ neutrophils expressed as a percentage of total $CD45^{+}$ white blood cells (WBCs). In both analyses, the optimal cut-off value providing the best balance between sensitivity and specificity for CML diagnosis was 2.0%, yielding a specificity of 100.0% (95% CI, 86.2% – 100.0%) and a sensitivity of 70.6% (95% CI, 46.9% – 86.7%).