Supplemental file 3

Methods of measurement of neutrophil CD64 expression and CRP

Blood samples were immersed in ice water and immediately transported to the laboratory for processing. Before each analysis of neutrophil CD64 expression, the QuantiBRITE PE beads (Becton Dickinson Immunocytometry Systems, San Jose, CA) conjugated with four predefined levels of phycoerythrin (PE) molecules were used to construct a standard linear regression curve. This quantitative method represents a substantial improvement over the measurement of relative fluorescence intensity, which may be easily affected by subtle, day-to-day operational and instrumental fluctuations. This means that the CD64 results in this study can be applied in other laboratories, increasing the generalizability of our findings. Freshly collected EDTA blood was maintained at 4°C and stained within 15 min after arrival at the laboratory. The CD64-PE antibodies were of QuantiBRITE grade (≥ 95% 1:1 antibody:PE ratio) and the staining procedures were performed according to the manufacturer's recommendation. We incubated 0.05 mL of whole blood with 20 μL of CD64-PE/CD45-peridinin chlorophyll antibodies (Becton Dickinson Immunocytometry Systems; cat. no. 340768) in the dark, at room temperature for 60 min. The red cells were then lysed with 1 mL of 1 × FACS Lysing solution (Becton Dickinson Immunocytometry Systems) for an additional 60 min before cytometric analysis. Thirty thousand events were acquired for each sample, using the FACSCalibur machine and CellQuest software (Becton Dickinson Immunocytometry Systems). The three parts of differential populations (lymphocytes, monocytes, and granulocytes) were identified and gated by their CD45/side-scatter profile. The expressions (geometric mean) of CD64 on neutrophils were measured quantitatively. The antibody-PE binding sites per cell were computed with QuantiQuest software (Becton Dickinson Immunocytometry Systems), using the linear regression curve of QuantiBRITE beads obtained in parallel with each sample analysis. This quantitative technique is both

accurate and reproducible between different laboratories. The distribution of fluorescence intensity is tightly clustered around the geometric mean in each subject. No subpopulations of CD64 negative neutrophils have been detected in any of our studies.

C reactive protein was measured by a turbidity assay kit against control standards, as specified by the manufacturer (Behring Diagnostics Inc., Westwood, MA).