Assessment of Neutrophil Function in Patients with Septic Shock: Comparison of Methods

C. WENISCH,1,2* P. FLADERER,2 S. PATRUTA,1 R. KRAUSE,2 AND W. HÖRL3

Division of Infectious Diseases, Department of Internal Medicine I,1 and Division of Nephrology, Department of Internal Medicine III,3 University Hospital of Vienna, Vienna, and Division of Infectious Diseases, Department of Medicine, University Hospital of Graz, Graz,2 Austria

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Patients with septic shock are shown to have decreased neutrophil phagocytic function by multiple assays, and their assessment by whole-blood assays (fluorescence-activated cell sorter analysis) correlates with assays requiring isolated neutrophils (microscopic and spectrophotometric assays). For patients with similar underlying conditions but without septic shock, this correlation does not occur.

Various neutrophil functions have been described as being reduced in sepsis, including adherence (21), chemotaxis (2), degranulation (20), phagocytosis (17), and production of reactive oxygen intermediates (ROI) (19, 24, 25). However, other studies have reported enhanced neutrophil chemotaxis and respiratory burst activities, particularly in patients without underlying conditions (8, 10, 12).

Neutrophils are considered fragile cells that are easily damaged by improper handling. Some tests of neutrophil function require isolation procedures to distinguish the effects of neutrophils on test results from those of other leukocyte types. These isolation procedures may be harmful to the cells or preactivate them.

In this study, we compared whole-blood flow cytometry assays (22) with fluorescence microscopy (13) and a cytochrome c reduction (5, 14) assay using Ficoll-Paque-separated neutrophils (11) for the assessment of neutrophil function in patients with septic shock and control subjects. In addition, killing capacity, levels of intracellular Ca2+, chemokinesis, and chemotaxis of neutrophils were evaluated.

The cases of 13 patients with septic shock (5 with APACHE II scores between 25 and 34) and of 13 subjects ranging in age from 26 to 84 years (mean ± standard deviation of 55 ± 18 years) with the same underlying conditions (coronary heart disease [n = 1], bladder carcinoma [n = 1], diabetes mellitus [n = 1], intravenous drug abuse [n = 2], chronic renal failure [n = 1], pancreatitis [n = 1], antiphospholipid antibody syndrome [n = 1], liver cirrhosis [n = 2], renal calculi [n = 1], abdominal trauma [n = 1], or no underlying condition [n = 1]) were investigated. No human immunodeficiency virus-positive patients were studied. Blood sampling was performed prior to antimicrobial, adrenergic, or steroid therapy. On routine differential counts, the septic neutrophils were all positive for toxic granulations.

All assays were performed blinded and were interpreted by S. Patruta and K. Stich. There was a >90% agreement between their readings.

*Corresponding author. Mailing address: Division of Infectious Diseases, Department of Medicine, University Hospital Graz, Auenbruggerplatz 15, A-8036 Graz, Austria. Phone: 43-316-385-2274. Fax: 43-316-385-4622. E-mail: christoph.wenisch@kfunigraz.ac.at.

Neutrophils were isolated from venous blood as described by Nauseef et al. (15) and Metcalf et al. (13). Phagocytosis and intracellular killing of opsonized Escherichia coli organisms were performed as described by Moiola (14), using E. coli strain ATCC 25922. Data are expressed as the percentage of bacteria phagocytized and killed by neutrophils. ROI production by neutrophils was determined by measuring superoxide dismutase-inhibitable reduction of cytochrome c according to the method of Nauseef et al. (15). Data are expressed as nanomoles of O2 produced by 2 × 106 cells. The calculation was made using the molar extinction coefficient of 29.9 × 103 mol/liter. The levels of intracellular Ca2+ in neutrophils were measured with Fura-2 AM, using fluorometer, model LS 5B (Perkin-Elmer, Norwalk, Conn.) according to the method of Alexiewicz et al. (1). Data are expressed as nanomoles per liter. Chemotaxis and chemokinesis levels were assessed using the under-agarose method (6). Levels of phagocytosis and ROI production by neutrophils were determined by flow cytometry according to the method of Wenisch and Graninger (22). All tests were performed in duplicate.

Differences between groups were calculated using the Student t test. Pearson’s correlation coefficient was used. All the analyses were two-sided, and differences with a P value of less than 0.01 were considered significant.

In patients with sepsis, the percentage of phagocytized bacteria, the number of E. coli organisms per neutrophil, and the percentage of killed bacteria were reduced (Table 1). In these patients, we found significant correlations between the level of phagocytosis, measured by fluorescence-activated cell sorting (FACS), and the percentage of phagocytized bacteria, measured by microscopic examination, (r = 0.784), and between the level of phagocytosis and the number of E. coli isolates per neutrophil (r = 0.748). The number of phagocytized bacteria that were killed was related to the level of stimulated ROI production, measured by the cytochrome c reduction assay (r = 0.735). No correlation between the FACS analysis results and the microscopic evaluations was seen for control subjects.

In sepsis, basal ROI production was increased, but the level of stimulated ROI production and the percentage of increase upon stimulation were decreased (Table 1). In septicemic patients, a correlation was seen between the basal ROI
Again, no relation between the FACS assay results and the between sepsis patients and control subjects (26 sepsis patients [5.001]). No relation between chemotaxis and intrinsic (auto-oxidation, etc.) to the neutrophils (22).

Altogether, this study suggests that both methods (i.e., using isolated neutrophils and using whole-blood-derived neutrophils) should be applied for an accurate interpretation of neutrophil function, particularly for patients with nonsevere depressed function.

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REFERENCES


