The Usefulness of an Inflammation Meter to Detect the Presence of Infection/Inflammation in Elderly Patients

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Background. Medical personnel who work in small geriatric institutions most frequently do not have access to real-time laboratory facilities.

Methods. In order to present a new method to determine the presence of an inflammatory response and for the assessment of its intensity, 118 patients aged 77±6 years with various bacterial infections were evaluated as well as 129 elderly individuals with various stressful conditions but no acute infections who served as controls. The leukocyte and erythrocyte adhesiveness/aggregation tests were performed by using a simple slide test and image analysis. The availability of the CD11b/CD18 and CD62L antigen on the leukocytes’ surface was measured by whole blood flow cytometry, and the quantitative C-reactive protein by using laser nephelometry and specific antihuman C-reactive protein antibodies.

Results. A significant difference was noted between patients and controls for all variables obtained by the slide test and image analysis. In addition, a highly significant correlation was noted between the number of leukocytes counted on the slides and white blood cell count, between the leukocyte adhesiveness/aggregation test and quantitative C-reactive protein, and between the degree of erythrocyte adhesiveness/aggregation and either the Westergren sedimentation or fibrinogen concentration.

Conclusions. By using our low-cost and real-time slide test, any medical or paramedical personnel can get relevant information regarding the presence of an acute phase response at the point of care.

METHODS

Patients and Controls

This prospective study was performed between January and April 1999 in the Department of Internal Medicine “D” at the Tel Aviv Medical Center, Israel, and was approved by the local ethics committee. Enrolled in the study were elderly patients (>66 years) with bacterial infection suspected by their clinical presentation in the first 48 hours of hospitalization and who met the study entry criteria. Included in the final data analyses were patients with bacterial infection further supported by the clinical condition and evidenced by positive blood, urine, stool, cerebrospinal fluid, or throat-swab cultures. Patients with lobar pneumonia (with appropriate clinical signs, symptoms, and chest x-ray) or those with skin and soft tissue infection were included as well. Although direct bacterial isolation was not possible in the latter, it is acceptable that the etiology of these infections is bacterial.

The control group consisted of the three following patient populations: (i) healthy individuals who were admitted for elective eye surgery; (ii) patients who were admitted for ob-
tween the aggregated red blood cells.

A quantitative measurement for the size of the spaces that form between the aggregated red blood cells is VR, which is denoted as vacuum radius. We also determined the degree of erythrocyte adhesiveness/aggregation.

The control group was composed of three populations because we wanted to compare elderly patients with similar stress profiles owing to the effects of acute disease or the hospitalization.

None of the controls had suffered from an infectious/inflammatory condition during the 3 months prior to the blood sampling. The control group patients were evaluated entirely during the first 24 hours of their stay in the hospital to prevent further changes in the peripheral blood due to a process of infarction or superimposed infections in the group with the stroke.

Excluded from the present study were individuals who had an underlying chronic infectious/inflammatory disease (such as chronic osteomyelitis or rheumatoid arthritis) or those with underlying malignancy. We also excluded patients who were receiving steroid or nonsteroidal anti-inflammatory medications (except for aspirin in a dose of <325 mg/daily). All patients and controls gave their informed consent for participation in the study.

**Laboratory Methods**

The WBCC and differential were performed with the Coulter STKS autoanalyzer (Coulter Electronics Ltd., Miami, FL), the ESR by using the method of Westergren, quantitative CRP concentrations by using laser nephelometry and FL, and the ESR by using the method of Westergren, quantitative CRP concentrations by using laser nephelometry and FL.

**Table 1. The Mean ± SD and Range of the Various Laboratory Data in Elderly Patients With an Acute Bacterial Infection and in the Age-Matched Control Group**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acute Bacterial Infection</th>
<th>Controls</th>
<th>t Test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count, cells per mm³</td>
<td>13.130 ± 6.120</td>
<td>7.780 ± 2.360</td>
<td>.0001</td>
<td></td>
</tr>
<tr>
<td>n = 116</td>
<td>n = 129</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Polymorphonuclears, %</td>
<td>83 ± 8</td>
<td>66 ± 9</td>
<td>.0001</td>
<td></td>
</tr>
<tr>
<td>n = 116</td>
<td>n = 128</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes, %</td>
<td>5.7 ± 3</td>
<td>8 ± 2</td>
<td>.0001</td>
<td></td>
</tr>
<tr>
<td>n = 116</td>
<td>n = 128</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>9 ± 6</td>
<td>23 ± 8</td>
<td>.0001</td>
<td></td>
</tr>
<tr>
<td>n = 116</td>
<td>n = 128</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>58 ± 33</td>
<td>27 ± 20</td>
<td>.0001</td>
<td></td>
</tr>
<tr>
<td>n = 114</td>
<td>n = 123</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>11.5 ± 7</td>
<td>0.9 ± 1.4</td>
<td>.0001</td>
<td></td>
</tr>
<tr>
<td>n = 102</td>
<td>n = 96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen, mg%</td>
<td>545 ± 148</td>
<td>274 ± 109</td>
<td>.0001</td>
<td></td>
</tr>
<tr>
<td>n = 97</td>
<td>n = 122</td>
<td></td>
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</tr>
</tbody>
</table>

**Note:** ESR = erythrocyte sedimentation rate; CRP = C-reactive protein.

Peripheral blood slides were prepared as previously described (7). They were scanned by using an image analysis system (INFLAMET™, Inflamet Ltd., Tel Aviv, Israel), the details of which were recently described (1–6). This system enables us to count the number of peripheral blood leukocytes per mm³ as well as determine their degree of adhesiveness/aggregation (percent of aggregated leukocytes) (Figure 1). In addition, the degree of erythrocyte adhesiveness/aggregation can be determined by measuring the “spaces” that are present between the aggregated red blood cells (8). A variable in the name of vacuum radius (VR) is created to represent the size of these holes (Figure 2).

Whole blood flow cytometry was performed by using FITC-labeled monoclonal antibodies to either CD11b/CD18 or the CD62L antigen as described (9). The monoclonal antibodies were purchased from Immunotech (Marseilles, France). The CD62L is an IgG1 monoclonal antibody (dreg 59) that recognizes the L-selectin antigen on the leukocytes.

**Table 2. The Mean ± SD and Range of the Various Inflammation Meter Variables in the Elderly Patients With an Acute Bacterial Infection and in the Age-Matched Control Group**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Acute Bacterial Infection</th>
<th>Controls</th>
<th>t Test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 118</td>
<td>n = 129</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l/mm²</td>
<td>498 ± 266</td>
<td>229 ± 97</td>
<td>&lt; .001</td>
<td></td>
</tr>
<tr>
<td>96–1722</td>
<td>22–546</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAAT %</td>
<td>28 ± 15</td>
<td>13.5 ± 7</td>
<td>&lt; .001</td>
<td></td>
</tr>
<tr>
<td>0–78</td>
<td>0–33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VR, microns</td>
<td>6 ± 8</td>
<td>2.4 ± 3</td>
<td>&lt; .001</td>
<td></td>
</tr>
<tr>
<td>0.5–39</td>
<td>0.3–23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** l/mm² = number of leukocytes per mm²; LAAT = leukocyte adhesiveness/aggregation test; VR = vacuum radius.
Figure 3. Representative pictures from patients with various degrees of inflammation. A, A control patient with a C-reactive protein level of 0.5 mg/l, a white blood cell count of 5200 per mm$^3$, polymorphonuclears of 52%, an erythrocyte sedimentation rate of 4 mm/h, a fibrinogen concentration of 243 mg/dl, an erythrocyte adhesiveness/aggregation test result of 0.6, and a leukocyte adhesiveness/aggregation test result of 0%. B, A patient (with chest pain) with C-reactive protein level of 0.5 mg/l, a white blood cell count of 7200 per mm$^3$, polymorphonuclears of 71%, an erythrocyte sedimentation rate of 16 mm/h, a fibrinogen concentration of 316 mg/dl, an erythrocyte adhesiveness/aggregation test result of 1.4, and a leukocyte adhesiveness/aggregation test result of 0%. C, A patient (with pneumonia) with a C-reactive protein of 12 mg/l, a white blood cell count of 14,300 per mm$^3$, polymorphonuclears of 83%, an erythrocyte sedimentation rate of 60 mm/h, a fibrinogen concentration of 681 mg/dl, an erythrocyte adhesiveness/aggregation test result of 5.8, and a leukocyte adhesiveness/aggregation test result of 26%. D, A patient (with sepsis) with a C-reactive protein of 21 mg/l, a white blood cell count of 11,100 per mm$^3$, polymorphonuclears of 82%, an erythrocyte sedimentation rate of 70 mm/h, a fibrinogen concentration of 751 mg/dl, an erythrocyte adhesiveness/aggregation test result of 22, and a leukocyte adhesiveness/aggregation test result of 74%.

The CD11b FIT is an IgGl antibody that recognizes the heterodimer glycoprotein of 165 KdA (a chain) and 95 KdA (b chain) (the MAC-1 antigen). Negative control for nonspecific flow cytometric background was performed with an isotype-matched, fluorescent, nonbinding monoclonal antibody.

Statistical Analysis

Correlations were calculated by using the Pearson correlation coefficient. Natural logarithm (Ln) transformation was performed on the vacuum radius parameter, CD62L and CD11b/CD18 MFI (mean fluorescence intensity) on polymorphonuclears, and CD11b/CD18 MFI on monocytes in order to achieve normal distribution of these parameters. The $t$ test was used to compare results of the conventional laboratory tests and those of the inflammation meter variables obtained in the bacterial and control groups. A $p$ value of $<.05$ was considered significant.

The statistical analysis was carried out using the SPSS (SPSS Inc., Chicago, IL) statistical package.

Results

A total of 132 patients with suspected bacterial infection were enrolled. Excluded were 11 patients who eventually turned out to be suffering from viral infection and 3 patients who turned out to have malignancy. Included in the study were 118 patients with an acute bacterial infection (aged $77 \pm 6$ years [mean $\pm SD$], 68 men and 50 women). Of them, 39 patients had pneumonia, 26 had urinary tract infection or pyelonephritis, 22 had soft tissue infections, 18 had sepsis, 10 had salmonella or shigella gastroenteritis, and the
The control group consisted of 21 individuals who were admitted for elective eye surgery (aged 75 ± 7 years, 10 men and 17 women), 59 who were admitted for chest pain observation and had no evidence of infarction (aged 74 ± 5 years, 41 men and 18 women), 49 individuals with an acute ischemic neurological event (aged 79 ± 7.5 years, 32 men and 17 women).

Using the t test, we compared the laboratory results of the study patients to those of the age-matched controls. There was no significant difference in the ages of the two groups. The results of the conventional laboratory tests showed a significant difference between the two groups (Table 1). A significant difference between patients and controls was also seen using the inflammation meter variables (Table 2). Representative pictures obtained from some of our patients with acute bacterial infections and from one control are given in Figure 3.

We then performed a correlation between the inflammation meter variables and the corresponding “conventional tests” for the entire study population. A highly significant correlation (r = .83, p = .0001, n = 245) was noted between the number of leukocytes per square mm by image analysis and the WBCC (Figure 4). Highly significant correlations were also found between the leukocyte adhesiveness/aggregation test and quantitative CRP (r = .5, p = .0001, n = 198) (Figure 5), between the VR and the ESR (r = .56, p < .001, n = 237) (Figure 6), as well as between the VR and the concentration of fibrinogen (r = .51, p = .01, n = 219) (Figure 7).

Finally, we report a significant difference between the groups in the expression of the leukocyte adhesion molecules CD11b/CD18 and CD62L obtained by whole blood flow cytometry (Table 3). There is an increased expression of CD11b/CD18 on the surface of both polymorphonuclear leukocytes and monocytes in the acute bacterial group compared to the control group. In addition, a significant correlation was found between the mean fluorescence intensity of the CD11b/CD18 antigen on the surface of peripheral blood polymorphonuclear leukocytes and quantitative CRP (r = .30, p < .001, n = 165). The correlation between polymorphonuclear CD11b/CD18 antigen and the leukocyte adhesive-aggregation test was also significant (r = .2, p < .001, n = 203).

**DISCUSSION**

We are in the process of evaluating a new diagnostic approach for the presence of an acute phase response. It is based on the known observations that this response is accompanied by the appearance of increased leukocyte numbers in the peripheral circulating pool of blood, increased leukocyte adhesiveness/aggregation, as well as enhanced synthesis of adhesive proteins (fibrinogen, for example) that can induce leukocyte as well as erythrocyte aggregation (10). Because all of these phenomena can be detected at real time and low cost by using a simple slide test (1–6,8), the next question is whether the diagnostic yield of our novel approach is comparable to what can be obtained by “conventional” markers, including the WBCC, erythrocyte sedimentation fibrinogen, or quantitative CRP concentrations. By using the same cohort of elderly patients, we could indeed show that the discrimination between the presence or absence of an inflammatory response in elderly patients with acute bacterial infections is as good as the one obtained by the above-mentioned conventional methods (6).

Having established the results obtained in children (11–13) and adults (14–16), we turned to the elderly population. This was done due to potential changes that can be observed with advancing age in white blood cell activity, expression and function of adhesion molecules, and in the pattern of cytokine production (17). In fact, it has been suggested that immunosenescence is associated with increased expression of several cell adhesion molecules resulting in augmented capacity to adhere (17) as well as increased production of proinflammatory cytokines. The results of the present study are similar to what can be observed in younger individuals with similar conditions of infection/inflammation (1–5,8),
suggesting that the usefulness of our diagnostic approach might not be different in the elderly population.

Stress accompanies acute bacterial infections, and the leukocytotic response might be affected by the individual stress response. The neuroimmuno-endocrinological response of an elderly patient might be different from the one observed in younger patients, and this was the reason we included stressful conditions like chest pain or acute neurological events in the control group. This enabled us to exclude the possibility that the changes observed in the patient group regarding leukocyte count (18) or adhesiveness/aggregation (19–21) are related to stress per se and not the bacteria-induced acute phase response.

In the present study, we evaluated patients with acute bacterial infections because of the significant therapeutic implications that are associated with their appearance in an elderly individual. In fact, acute bacterial infections are among the leading causes of mortality in this age group. The prompt identification of such an infection might therefore be relevant for the prompt administration of antibiotics, thus improving the chances for an eventual recovery. It has been repeatedly shown by several groups of researchers that bacterial infections are associated with the appearance of increased leukocyte adhesiveness/aggregation (15,16,22,23) and that the degree of this adhesiveness/aggregation correlates with the intensity of the inflammatory response (9,14,24,25). In addition, this adhesiveness/aggregation is probably much more attenuated in acute viral infections (11–13,26) suggesting the eventual possibility of singling out patients with acute febrile conditions who are at risk of having acute bacterial infections. The reasons for the appearance of an enhanced leukocyte adhesiveness/aggregation are probably complex and related to both the expression of adhesion molecules on the surface of peripheral blood leukocytes (16,22) as well as increased concentrations of adhesive plasmatic proteins (27). The results reported here regarding the expression of the CD11b/CD18 antigen are in accordance with what has been reported in the past for younger individuals (16,22).

We have recently completed the development of software that will permit the identification of the same information without the need to stain the slides. This will enable para-medical personnel to obtain the results of the test within less than 10 minutes. In addition, by using modern telemedicine facilities, they will be able to compare the picture to a relatively large number of matched controls as well as to the results of the patient himself or herself during remission. Images can be transferred via the Internet to the attending physicians not present on scene. The costs are extremely low and, depending on the number of tests performed per system, are estimated to be as low as $5 per test.

We conclude that it is possible to use a simple slide test and image analysis to reveal relevant information related to the acute phase response. We presented evidence that this approach is applicable to elderly individuals with acute bacterial infections. Our technology might be welcomed by medical teams who are involved in the care of elderly patients who reside in small centers and institutions where
bedside laboratory facilities are not available at real time and low cost.

Acknowledgment

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References


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