Nitroblue Tetrazolium Reduction:
False Positive and False Negative Results

By Philip Ashburn, M. Robert Cooper, Charles E. McCall, and Lawrence R. DeChatelet

Quantitative and histochemical nitroblue tetrazolium reduction (NBT) tests were performed on leukocytes of 13 patients with polycythemia vera, seven with chronic granulocytic leukemia, eight with neoplastic disease associated with fever, 16 with bacterial infection, and 13 healthy control individuals. No significant differences were detected in the quantitative test between any of the groups studied. The histochemical NBT test was significantly higher than control in the patients with polycythemia vera and neoplasia associated with fever, as well as in those with known bacterial infection. It is suggested that patients with neoplasia or polycythemia vera may show a false positive reaction in the histochemical NBT test. Conversely, the test was significantly lower than control in patients with chronic granulocytic leukemia. Such patients might show a false negative reaction in the test even if infection were present.

During the process of phagocytosis by the polymorphonuclear leukocyte (PMNL), there occur significant increases in oxygen consumption, hexose monophosphate shunt activity, and hydrogen peroxide formation.1 The importance of these reactions to the bactericidal process is deduced primarily from patient studies in which the cells fail to respond metabolically following particle ingestion.2,3 Such cells are characterized by defective bactericidal activity, and the patients suffer from severe recurrent infections.

Although the precise nature of the enzymatic reaction that initiates these events is controversial, the enzyme may be related to a diaphorase that can reduce nitroblue tetrazolium (NBT) dye to the insoluble blue formazan.4 PMNL from patients that fail to show the usual “respiratory burst” on phagocytosis likewise fail to reduce NBT.3,4 This observation led Baehner and Nathan to develop a quantitative assay for NBT reduction that could be used as a screening test to detect patients with chronic granulomatous disease and carriers of the disease.5

Attention has recently focused on a histochemical NBT test in which the percentage of resting cells containing formazan deposits is determined;6 this

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test has been suggested as an aid in the differential diagnosis of bacterial infection.

The present communication compares values for the quantitative and histochemical NBT tests in leukocytes from control subjects and from patients with a number of hematologic and neoplastic diseases.

MATERIALS AND METHODS

Five groups of patients were studied; these included normal controls, patients with polycythemia vera, patients with chronic granulocytic leukemia, patients with neoplastic disease associated with fever, and patients with acute bacterial infection.

Differentials were done in the counting chamber by classifying cells as phagocytes (mature and band form neutrophils, eosinophils, and monocytes) and lymphocytes. Wright-stained differentials were also performed on all subjects.

Nitroblue Tetrazolium Reduction

Quantitative NBT reduction was measured by the method of Baehner and Nathan\(^5\) in which the reduced dye is extracted with pyridine and measured spectrophotometrically. Values are expressed as absorption at 515 nm/15 min/2.5 × 10\(^6\) cells.

The histochemical reduction of NBT was performed by the method of Matula and Paterson.\(^7\) Only neutrophils with cytoplasmic clumps of formazan deposit were considered positive. Results were expressed as per cent positive after counting 100 neutrophils.

RESULTS

Table 1 gives the differential counts obtained on Wright-stained blood smears. The presence of lymphocytes does not affect the quantitative NBT test, since the white cell suspension is based on a known number of phagocytes. Similarly, the number of immature forms seen in chronic granulocytic leukemia and bacterial infection should not affect the results for the same reason. Since the histochemical NBT test scores only PMNL containing formazan, the value obtained for a patient is independent of the differential count. The histochemical reduction of NBT was not observed in lymphocytes or neutrophils less mature than the band-form cell. Monocytes actively reduced the dye.

The results of the NBT tests are shown in Table 2. The \(p\) values listed were derived using the F test and Student’s t test to compare the variance and mean of each patient population to that of the corresponding control population.

The data show that none of the groups differed significantly in the quantitative NBT test in either the resting or phagocytizing state. In the histochemical NBT test, however, each disease population differed significantly from the control population \((p < 0.001)\). Noninfected patients with polycythemia vera or neoplastic disease with fever had a significant elevation in the percentage of NBT positive PMNL compared to the control subjects. As expected, cells from infected patients showed a markedly elevated histochemical NBT test as compared to those from the control group. On the other hand, cells from patients with chronic granulocytic leukemia had a significantly lower histochemical NBT test than those from the control population.
<table>
<thead>
<tr>
<th></th>
<th>Control N = 13</th>
<th>Polyglobulia N = 13</th>
<th>Hypereosinophilia N = 7</th>
<th>Chronic Granulocytic Leukemia N = 7</th>
<th>Neoplasia N = 8</th>
<th>Infection N = 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC</td>
<td>7500 ± 2500</td>
<td>6800 ± 1500</td>
<td>41 ± 23</td>
<td>75 ± 14</td>
<td>64,900 ± 41,000</td>
<td>7400 ± 2500</td>
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<tr>
<td>Differential</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Eosinophils</td>
<td>50 ± 10</td>
<td>68 ± 7</td>
<td>1 ± 0.7</td>
<td>7 ± 0.3</td>
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<td>Basophils</td>
<td>2 ± 1</td>
<td>1 ± 0.5</td>
<td>0.1 ± 0.2</td>
<td>1 ± 0.5</td>
<td>0.1 ± 0.2</td>
<td>1 ± 0.5</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>30 ± 10</td>
<td>41 ± 7</td>
<td>16 ± 14</td>
<td>17 ± 10</td>
<td>15 ± 10</td>
<td>16 ± 14</td>
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<tr>
<td>Monocytes</td>
<td>6 ± 2</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
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<td>5 ± 1</td>
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<tr>
<td>Eosinophils</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
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<tr>
<td>Neutrophils</td>
<td>2 ± 1</td>
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<tr>
<td>Band cells</td>
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<td>Nucleated RBC</td>
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<td>0 ± 1</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>Total</td>
<td>7500 ± 2500</td>
<td>6800 ± 1500</td>
<td>41 ± 23</td>
<td>75 ± 14</td>
<td>64,900 ± 41,000</td>
<td>7400 ± 2500</td>
</tr>
</tbody>
</table>

*Number of patients in group; †All values are mean ± SD.

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Table 2. NBT Reduction by Leukocytes From Various Patients

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Quantitative NBT</th>
<th>Histochemical NBT†</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Resting</td>
<td>Phagocytizing</td>
</tr>
<tr>
<td>Control†</td>
<td>0.099 ± 0.033</td>
<td>0.279 ± 0.091</td>
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<tr>
<td>Polycythemia vera</td>
<td>0.107 ± 0.046</td>
<td>0.285 ± 0.063</td>
</tr>
<tr>
<td>Chronic granulocytic leukemia</td>
<td>0.105 ± 0.049</td>
<td>0.265 ± 0.065</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>0.083 ± 0.039</td>
<td>0.219 ± 0.065</td>
</tr>
<tr>
<td>Bacterial infection</td>
<td>0.071 ± 0.021</td>
<td>0.331 ± 0.043</td>
</tr>
</tbody>
</table>

*Absorption 515 nm/15 min/2.5 X 10⁶ phagocytes.
†Per cent of phagocytes positive for formazan.
‡Values = mean ± SD; N = number of patients; p value compares patient cells to corresponding control.

DISCUSSION

The discrepancies between the quantitative and histochemical NBT tests are disturbing, but others have likewise reported difficulty with the quantitative assay. The lack of correlation between the two tests may be the result of procedural differences, or they may reflect differences in cell population.

The results of the histochemical NBT test are of some interest. This test has been reported elevated in systemic bacterial and fungal disease, as well as in malaria, tuberculosis, and other parasitic diseases. Thus, this test has been suggested as a diagnostic aid in systemic infections. However, an elevated histochemical NBT test has been described in some patients without systemic infection. Park et al. have reported elevated values in noninfected infants under 2 mo of age. Reference has likewise been made to the fact that noninfected patients with the triad of Hodgkin’s disease, fever, and neutrophilic leukocytosis have elevated histochemical NBT values. The present communication adds two more groups of false positive tests by demonstrating abnormally high values in patients with polycythemia vera and neoplasia associated with fever. One must recognize that in these diseases an elevated histochemical NBT test is not necessarily an indication of the presence of systemic bacterial infection.

On the other hand, a false negative test may be encountered in infected patients with chronic granulomatous disease, and lipochrome histiocytosis, or in patients on steroid therapy. The abnormally low values observed here in the leukocytes of patients with chronic granulocytic leukemia suggest that a false negative test might be encountered in this condition as well. We have not yet had an opportunity to test this hypothesis by studying a patient with chronic granulocytic leukemia during an acute bacterial infection.
ACKNOWLEDGMENT

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REFERENCES

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