The Nitroblue-Tetrazolium Test in the Investigation of Febrile Patients

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SUMMARY

The nitroblue-tetrazolium (NBT) test was performed on 71 hospitalized febrile patients and compared with 66 afebrile control patients. A level of 17% NBT-positive polymorphonuclear cells per 100 leucocytes or higher, was selected to indicate the presence of bacterial infection. In 20 subjects with proved, but untreated, bacterial infection, all but 2 had elevated NBT levels. Nine patients with bacterial infections, who had received prior antibiotic or steroid therapy, had NBT values within the normal range. A false-positive reaction was observed in 24% of febrile subjects who were diagnosed as suffering from non-bacterial infection. In spite of the high false-positive incidence, the NBT test is still of clinical use, provided that the subjects are carefully selected.


Physicians have long sought a simple, rapid and reliable method for the diagnosis of bacterial infections. In 1968 Park et al. described the spontaneous reduction of nitroblue-tetrazolium (NBT) by neutrophils, as an aid in the diagnosis of bacterial infection. The usefulness of this test has been confirmed by other authors in the diagnosis of bacterial, malarial, systemic fungal and helminthic infections. Thus, the NBT test has been proposed as a routine screening procedure in the evaluation of febrile patients. Recently, however, a large number of both false-positive and false-negative findings have been reported. Because of this criticism, it was decided to further evaluate the NBT test and to investigate its usefulness for the clinician in the diagnosis of the pyrexial patient.

MATERIALS AND METHODS

Subjects Studied

A total of 137 patients was studied. The control group consisted of 66 non-febrile patients admitted to hospital for reasons other than suspected bacterial or viral disease. All were free of infection or haematological disorder, and included patients with ischaemic, rheumatic and hypertensive heart disease, as well as a number of healthy hospital personnel. The average value, range, standard deviation (SD) and standard error of the mean (SE) of the NBT test values were calculated for this group to serve as normal reference values for our specific working conditions. Another group of subjects was made up of 71 febrile patients (children and adults).

Technique

The NBT dye test was performed according to the method proposed by Park et al., as modified by Matula and Paterson. The test was carried out on whole venous blood, not later than 30 minutes after collection of the blood. During the early test performance both EDTA and heparin were used as anticoagulants. Significantly lower results were consistently obtained with EDTA, a result confirmed by Freeman and King, so EDTA was abandoned.

Calculation of Results

Some authors include the NBT-positive monocytes in the count, while others exclude monocytes. In this study the NBT-positive monocytes were not included. It was considered a more logical approach to express the result by calculating the number of NBT-positive polymorphonuclear leukocytes (PMN) as a percentage of the total white blood cell count. This value was obtained by multiplying the mean percentage of NBT-positive PMN obtained from at least 200 PMN cells, with the percentage of PMN-leucocytes obtained in the usual way through a differential count of the Giemsa-stained smear. The result was expressed as NBT-positive PMN-leucocytes per cent of white blood cells. Moreover, it must be stressed that cells were considered as NBT-positive only when they contained definite massive or particulate blue-black deposits of reduced NBT within their cytoplasm.

RESULTS

The results are summarized in Table I and illustrated in Figs. 1 and 2. A total of 137 patients was studied. For the 66 control subjects, the mean NBT value obtained was 8.3 (range 2.2 - 19.4; SD ± 4.17; SE ± 0.51). Accordingly, an arbitrary score of 17 and above was selected to indicate the presence of bacterial infection. This level is considerably higher than that of Park, but has been confirmed by other authors.
An analysis of febrile patients was made from a clinical point of view. They were divided into 3 distinct groups. Group A consisted of 29 patients with proved bacterial infection or malaria, who had received no prior chemotherapy or steroids. Group B consisted of 9 patients with bacterial infection, already receiving substantial therapy, and group C of 33 febrile patients with causes other than bacterial infection, malaria, helminthic or systemic fungal infestation (Fig. 2).

In group A the mean NBT value was 26.9 (range 4.9 - 50.4; SD ± 9.2; SE ± 1.7); whereas in group B, after therapy, the mean NBT score was 8.7 (range 3.4 - 13.7; SD ± 3.5; SE ± 1.1). In group C, febrile patients due to non-bacterial aetiology, the mean value was 10.7 (range 0.6 - 28.2; SD ± 8.3; SE ± 1.4).

When the results were evaluated from a laboratory point of view, a similar profile was observed. There were

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**TABLE I. RESULTS OF NBT ESTIMATION**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Bacterial infections, no therapy</th>
<th>Bacterial infections, treated</th>
<th>Non-bacterial cause of fever</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>66</td>
<td>29</td>
<td>9</td>
<td>33</td>
</tr>
<tr>
<td>Average value</td>
<td>8.3</td>
<td>26.9</td>
<td>8.7</td>
<td>10.7</td>
</tr>
<tr>
<td>Range</td>
<td>2.2 - 19.4</td>
<td>4.9 - 50.4</td>
<td>3.4 - 13.7</td>
<td>0.6 - 28.2</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>±4.1</td>
<td>±9.2</td>
<td>±3.5</td>
<td>±8.3</td>
</tr>
<tr>
<td>Standard error</td>
<td>±0.5</td>
<td>±1.7</td>
<td>±1.1</td>
<td>±1.4</td>
</tr>
</tbody>
</table>

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Fig. 1. NBT estimation in control subjects. Average value illustrated by horizontal line.

Fig. 2. NBT estimation in febrile patients. A—untreated bacterial infections; B—treated bacterial infections; C—non-bacterial febrile patients. Average values illustrated by horizontal lines.
40 febrile patients with elevated NBT scores. Of these patients, 3 were excluded because of an inconclusive final diagnosis. Of the 37 remaining subjects, 25 were shown to have proved bacterial infection; a further 2 had Plasmodium vivax malaria. Thus, only 76% of febrile patients with elevated NBT scores had bacterial or malarial infections, i.e. 24% of the patients with elevated NBT scores were febrile due to non-bacterial, non-fungal, or parasitic infections. Analysis of these false-positive reactions included isolated cases of aseptic meningitis, encephalitis, infectious hepatitis, cat-scratch fever, Mycoplasma pneumoniae, and 2 cases of rheumatic carditis. The findings of 24% false-positive scores is highly significant.

Evaluation of 35 patients with normal NBT scores showed a variety of conditions, including 5 patients with infectious hepatitis, 2 with Dressler’s syndrome, 7 with infectious mononucleosis, 2 with Hodgkin’s disease, 1 with heat-stroke and, most commonly, viral upper respiratory tract infections. Included in this group, however, were 9 patients with proved bacterial infections, all of whom had received considerable quantities of antibiotics or steroids prior to NBT measurement. In addition, 2 patients, 1 with pneumococcal pneumonia and 1 with typhoid fever, were seen. They had not received antibiotic therapy (true false-negative). Of the 27 febrile cases due to untreated bacterial infections, all but 2 had elevated NBT scores, whereas prior treatment with steroids and antibiotics virtually always suppressed the NBT score (Fig. 2).

**DISCUSSION**

There is a need for a reliable and rapid test to confirm systemic bacterial infections. Leucocytosis, toxic granulations, a shift to the left, when present, are all suggestive, but not diagnostic of bacterial infection. Their absence by no means excludes bacterial infection. Park introduced the NBT test as a simple, readily applicable test which could greatly assist the physician faced with the dilemma of a febrile patient. Subsequent reports supported the clinical usefulness of this laboratory test.

While few physicians doubt the value of this test for the diagnosis of chronic granulomatous disease of infancy, a number of authors have shown less enthusiasm with the results of the NBT evaluation because of many false-positive and false-negative results. Furthermore, the test is not applicable in a growing number of clinical situations.

It may be said that NBT estimation is of little value in patients with profound haematological disorders, impaired immune mechanisms, and following steroid, immunosuppressive or antibiotic therapy. The clinical value of this test is therefore considerably reduced when many subjects with the above-mentioned disorders are at the risk of developing superimposed infections. Nevertheless, patients after open-heart surgery may be monitored by daily NBT estimations in order to determine the early diagnosis of bacterial infection, especially when intravenous catheters are used.

Numerous reports of false-positive reactions have appeared in the literature. These include infections in newborn infants, the Chediak-Higashi syndrome, typhoid/paratyphoid vaccinations, myelofibrosis, mycoplasma infections, osteogenesis imperfecta, and viral meningitis due to ECHO virus, Coxsackie viruses, herpes and mumps virus infections. In this investigation 24% of the febrile subjects without detectable bacterial infection had elevated NBT levels. These include active rheumatic carditis, infectious hepatitis, carcinoma of the colon, encephalitis, and aseptic meningitis. This high figure is of considerable significance, and in no small way detracts from the clinical usefulness of the test.

Similarly, false-negative results have been reported in infective endocarditis, bacterial meningitis, Salmonella, and urinary tract infections. In this series only 2 out of 29 untreated subjects febrile due to bacterial infections had normal scores. It is noteworthy that in all 9 infected febrile patients who were receiving antibiotic or steroid therapy, normal results were obtained. False-negative results have been reported with localized infection such as cystitis and cellulitis, but all our subjects had pyrexia.

In the presence of leucopenia, the neutrophil-reductive ability was in no way impaired, and the 2 patients with hypersplenism with associated bacterial infection had elevated NBT estimations.

The finding of a positive NBT result in tertian malaria was confirmed in 2 cases.

It may be concluded that while the NBT dye test may yet be of value to the clinician, results require cautious interpretation with attention being given to the therapy received as well as to the haematological and immune status of the patient. The NBT test is of additional use in the early diagnosis of bacterial infection in the susceptible individual, and, moreover, a decreasing NBT value even in the absence of clinical change may reassure the physician of early clinical improvement. Another use for the NBT test may well be in a case of clinical meningitis where the NBT estimation of specimens of cerebrospinal fluid may indicate the aetiology of the infection.

**REFERENCES**