The Granulocyte Nitroblue Tetrazolium Reductase Test

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ABSTRACT

The nitroblue tetrazolium (NBT) test is most useful for the diagnosis of chronic granulomatous disease and a few other rare metabolic abnormalities of neutrophils. It may be helpful in suggesting the presence of infection when values of both percentage of NBT positive neutrophils and total number of NBT positive neutrophils are markedly increased. Each patient must be interpreted with cautious attention to the primary disease and other clinical and laboratory findings. Diseases associated with necrosis, such as malignancies and myocardial infarction, will often cause elevation of neutrophil count and increased conversion of NBT. The NBT test may be useful in differentiating acute monocytic leukemia from acute myeloblastic leukemia.

Introduction

Nitroblue tetrazolium (NBT) is one of a series of tetrazolium salts and is the most sensitive of the series in detecting dehydrogenase activity. Its structure is 2,2' di-p-nitrophenyl-5,5' diphenyl-3,3' (3,3' dimethoxy-4,4' biphenylene) ditetrazolium chloride (figure 1). It is a light yellow salt which develops a blue black precipitate (formazan) when reduced.

Although it had been utilized in several studies of cardiac and other tissues to localize dehydrogenases, Baehner and Nathan were first to use the dye in the study of dehydrogenase activity in blood neutrophils in 1967. Since then the test has been used to diagnose chronic granulomatous disease (CGD) and to aid in the differentiation of bacterial infection from neoplastic conditions and inflammatory states. It has been suggested the test might aid in the differentiation of acute monocytic leukemia.

The work of Baehner and Nathan suggested that CGD depended upon the lack of an NADH oxidase* responsible for the production of the H₂O₂ necessary to the halogenation and killing of phagocytized bacteria. In the case of NADH oxidase deficiency NBT could not be reduced and hence the NBT test could be used as a

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* Nicotinamide adenine dinucleotide oxidase.
screening test for metabolic deficiency of the neutrophils.

Holmes, Page, and Good⁵ were quick to argue that since their patients with CGD appeared to have normal NADH and NADPH oxidase activity, that deficiency of other dehydrogenase systems could be the basis for the lack of the respiratory burst usually associated with phagocytosis, and the inability of CGD neutrophils to kill catalase positive Staphylococcus aureus, E. Coli, or Serratia Marcescens.

Humbert et al⁶ showed that methylene blue (MB) would reduce NBT in the presence of NADPH⁺ and NADH but not with GSH⁻ in vitro cell free preparations. Whether or not in vitro MB stimulates enzymes such as G6-PD,⁶ GSSG reductase,⁷ GSH peroxidase, and NADH oxidase, which presumably transport electrons to NBT in resting and phagocytizing cells, is still unknown.

Mandell et al⁸ showed that the NADH oxidase inhibitor, hydrocortisone, also inhibited intraneutrophil killing of catalase positive bacteria. Phagocytosis and degranulation appeared normal as judged by appearance under the electron microscope and acid phosphatase release. After phagocytosis, hydrocortisone-treated neutrophils demonstrated less NADH oxidase activity, oxygen consumption and hydrogen peroxide production. In addition, NBT dye reduction was diminished in hydrocortisone treated neutrophils. Thus the evidence grew that NBT reduction depended upon NADH oxidase and that CGD resulted from a deficiency of this enzyme.

In 1968, Park et al¹¹ suggested that spontaneous NBT reduction might occur in the neutrophils of patients with infection and proceeded to demonstrate a marked difference from normal in both the percentage of

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¹ Nicotinamide adenine dinucleotide phosphate.
² Reduced glutathione.
⁶ Glucose—6 phosphate dehydrogenase.
⁷ Glutathione reductase.

NBT positive neutrophils and the total number of NBT positive neutrophils in patients with proven bacterial infections. Matula and Paterson modified the test by prolonging incubation at 37° to 25 minutes, by counting stippled cells as well as cells with large clumps of formazan as positive, and by utilizing only the percentage of NBT positive neutrophils as the criteria for infection.⁹ This practice resulted in a number of false positives and false negatives. Confusion in the literature as to the reliability of the test has resulted. Feigin et al⁴ developed a nomograph which includes the total number of NBT positive neutrophils on the ordinate and the percentage positive neutrophils on the abscissa. By using both parameters, they indicated the patient could be classified normal (A), inflammatory disease or viral infection (B), bacterial infection (C) or severe nonresponding infection (D). By this method, they suggested many of the so-called false negatives could be avoided.

Vickers and Hayes provided further evidence for this approach.¹⁵ They demonstrated in 100 patients that the total white
count and number of bands had no correlation with the percent of NBT positive cells, but the total number of bands correlated with the white count. They pointed out that leukocytosis and left shift occur as a kinetic phenomenon dependent upon rapid release of cells from the stimulated bone marrow. The NBT reaction, however, appears to depend upon direct stimulation of the neutrophil oxidative metabolism by bacterial products. Thus, use of the two parameters might be more reliable in assessing the presence of infection.

NBT Test in Hematological Disorders and Malignancies

In assessing the NBT test in various malignant conditions, several reports indicate that fever and an elevated NBT score can be seen in lymphomas and Hodgkin's disease and in the myeloproliferative diseases. It should be emphasized that each of these authors used only the percent NBT positive neutrophils to determine whether a patient should be scored as normal or infected. Furthermore, Ashburn suggested patients with chronic granulocytic leukemia (CGL) might show falsely low NBT scores in the face of infection. This is far from proven. A patient with a white count of 100,000 with a score of 5 percent NBT positive neutrophils may have infection, since the total neutrophils positive will be 5,000, ten times that usually seen in the normal individual.

Most of the studies now in the literature do not provide raw data, and thus it is impossible to determine whether or not the patients could be more appropriately classified by the Feigin nomograph. Ashburn provided some data for patients with various hematological and other malignancies. If their data (table I) are superimposed upon the chart of Feigin (figure 2), most of the values in these noninfected patients fall within the normal or inflammatory categories A and B. The notable exception is the group with other than hematological tumors labelled neoplasia. These eight patients had values close to or in the infected range (category C). The eight patients with neoplasia had average white counts of 7,400 ± 2,500 with 75 percent neutrophils, 29 percent of which were NBT positive.

The possibility exists that these patients actually did have infection which was clinically undetected but the patients with proven infection had much higher average counts—WBC of 15,000 with 50 percent neutrophils, 15 percent band forms and 37 percent NBT positive (table I). Thus, it seems more likely that tumor necrosis might stimulate moderate neutrophil response, whereas infection produces a more marked response in these parameters.

Lauter et al demonstrated that factors released from necrotic myocardial tissue can induce neutrophilia and substantial NBT reduction in circulating neutrophils. These authors were able to demonstrate in 14 patients with EKG proven infarcts that the NBT reduction was increased both in percent and in total number of neutrophils (figure 2).

| TABLE I |
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| No. | NBT | **NBT Pos.** |
| Ashburn Study | Blood 1973 |  |
| Normal | 13 | 6.6±1.0 | 259±150 |
| Polycythemia vera | 13 | 24.0±10.0 | 1152±700 |
| Chronic granulocytic leukemia | 7 | 3.0±2.0 | 2050±1000 |
| Neoplastic disease | 8 | 29.0±13.0 | 1620±1000 |
| Infection | 16 | 37.0±4.0 | 3700±1500 |
| Myocardial infarction | 14 | 30.0±10.0 | 2348±1000 |

*Total NBT positive neutrophils were calculated from WBC x percent neutrophils and bands x percent NBT positive neutrophils and bands.*
Thus there is sufficient overlap in NBT scores as to render the test of questionable value in differentiating infection in these states. It seems likely that both bacterial products and products from necrotic tumor tissue or damaged myocardial tissue may induce neutrophilia left shift and increased NBT reduction. The severity of the disease may be a greater influence on the degree of NBT reduction than the specific disease.

It still seems valid to conclude that high NBT scores by both parameters points toward the presence of infection in chronic granulocytic leukemia and polycythemia vera. Furthermore, very high values in total number of NBT positive neutrophils must make one more suspicious of bacterial infection in other disease conditions than low values would.

Finally, Catovsky has indicated a good correlation between the NBT score in monoblasts, their content of lysozyme and the serum lysozyme level in monocytic leukemia. Ten patients with acute myeloblastic leukemia by morphologic criteria, and low serum lysozyme level, had very few positive cells (0 to 1 percent). Similarly, eight patients with morphologic criteria of acute monomyelocytic leukemia but with low serum lysozyme levels had low levels of NBT positivity (0 to 13 percent with an average of 6 percent). In contrast, five patients with acute monomyelocytic leukemia and three patients with acute monocytic leukemia with high serum lysozyme levels had NBT percentages of 6 to 56 percent with an average of 33 percent positive blast forms. There was also a correlation between NBT positivity and cellular lysozyme positivity. It may be that monoblasts develop their NBT reducing enzymes (NADH oxidase) earlier than the myeloblasts do, thus allowing for a differential staining pattern. The number of patients thus far studied is small and the results must be confirmed.

References


