Nitroblue Tetrazolium Test in the Diagnosis of Pleural Effusions*

Richard W. Light, M.D., F.C.C.P.; and Ronald B. George, M.D., F.C.C.P.

This study evaluated the diagnostic utility of the nitroblue tetrazolium test on pleural fluid. Pleural fluids from 62 patients, including nine with congestive heart failure, 27 with malignant neoplasms, nine with tuberculosis, 14 with pneumonia, and three with collagen vascular disease, were studied. Results of nitroblue tetrazolium were tabulated for each patient for three different cellular types (polymorphonuclear leukocytes, small lymphocytes, and mononuclear cells other than small lymphocytes). The three patients with collagen vascular disease had very high scores on the nitrogen tetrazolium test for all three cellular types. Their average scores were higher than were those of 57 of the other 59 patients. The nitroblue tetrazolium test on pleural fluid was not useful in separating patients with congestive heart failure, pneumonia, malignant neoplasms, or tuberculosis. The results on the nitroblue tetrazolium test did not help to identify those patients with pneumonia who eventually required tube thoracostomy. From this study, we conclude that the nitroblue tetrazolium test on pleural fluid is of limited use diagnostically but may help to identify those individuals with collagen vascular disease involving the pleura.

The nitroblue tetrazolium test involves the incubation of white blood cells with nitroblue tetrazolium dye. Leukocytes from patients with bacterial infections more readily phagocytize and reduce nitroblue tetrazolium than do leukocytes from noninfected individuals. This is the basis of the proposal by Park et al for using the test to separate patients with bacterial infections from those without bacterial infections. Indeed, an editorial in 1971 suggested that the test should become a routine screening procedure for detecting systemic bacterial infections or for excluding viral or other noninfectious diseases; however, subsequently, it was shown that for the nitroblue tetrazolium test on blood, there is much overlap between the results on patients with bacterial infections and patients febrile from other causes.

Nevertheless, the nitroblue tetrazolium test has proved useful in diagnosing bacterial infections in fluids other than serum. Gupta and Steigerwald reported that patients with pyogenic arthritis had higher results of the nitroblue tetrazolium test on their synovial fluid than did patients with nonpyogenic arthritis. These investigators conclude that the nitroblue tetrazolium test on synovial fluid was a simple and useful method for the early detection of pyogenic arthritis. The objective of the present study was to assess the usefulness of the nitroblue tetrazolium test on pleural fluid in the differential diagnosis of pleural effusions.

Materials and Methods

The patients in this study were seen at the Louisiana State University Medical Center in Shreveport in 1976 and 1977. A total of 62 patients were studied, including patients with congestive heart failure, pleural malignant neoplasms, pleural tuberculosis, pneumonia and effusion, systemic lupus erythematosus, and rheumatoid pleuritis. The criteria for the various diagnostic categories are the same as those that we have used previously.

The nitroblue tetrazolium test was adapted for pleural fluid from the method of Park et al. Crystalline grade-3 nitroblue tetrazolium dye (Sigma Chemical Co.) was dissolved as a 0.2 percent solution in 0.15M phosphate-buffered saline solution (pH 7.2). The pleural fluid was collected in a plastic syringe to which EDTA was added to prevent coagulation. It is important not to use glass syringes or heparin because both stimulate the cells to phagocytize the nitroblue tetrazolium.

In order to concentrate the cells in the pleural fluid, approximately 10 ml of pleural fluid was centrifuged, and the sediment was subsequently resuspended in 1.0 ml of supernatant. Ten drops of the fluid were gently mixed with ten drops of the nitroblue tetrazolium solution, and the mixture was incubated for 45 minutes at 37°C. At the end of the incubation, resuspension of the cells was accomplished by gentle agitation. One drop of the suspension was placed on a glass slide and smeared, as in performing a smear of peripheral blood. After drying, the preparation was counterstained with a 1 percent aqueous solution of safranin for two minutes.

In order to quantitate the nitroblue tetrazolium test, the cells in the pleural fluid were separated into the following three categories: (1) polymorphonuclear leukocytes; (2) small lymphocytes; and (3) mononuclear cells other than

*From the Departments of Medicine, Veterans Administration Medical Center, Long Beach, the University of California, Irvine, and Louisiana State University, Shreveport. Presented in part at the 44th Annual Meeting, American College of Chest Physicians, Washington, DC, Oct 29-Nov 2, 1978.
Reprint requests: Dr. Light, VA Medical Center, Long Beach, California 90802
small lymphocytes. In the pleural fluid the latter category included mesothelial cells, macrophages, plasma cells, large lymphocytes, and malignant cells. The amount of nitroblue tetrazolium taken up by each cell was quantitated as follows: 0, negative (no nitroblue tetrazolium taken up); 1, slightly positive (one to five small granules); 2, moderately positive (six or more small granules); and 3, strongly positive (large numbers of small or one or more large granules).

If present, 100 cells in each of the three categories were counted. The score for each category of cells was the sum of the scores for the 100 cells in that category. The average score for a patient was the average of the scores for the individual categories. Since in 27 patients, cells from one category were absent and since in another three patients, cells from two categories were absent, the average score in these 30 patients was taken as the average of the scores for the categories present. In Figures 1 to 3 no point is included for a patient if cells were lacking in that category.

RESULTS

Pleural fluid from 62 different patients was studied. The diagnoses for these 62 patients were as follows: congestive heart failure, 9; pleural malignant neoplasms, 27; tuberculous pleuritis, 9; pneumonia with effusion, 14; and collagen vascular disease, 3 (systemic lupus erythematosus, 2; and rheumatoid disease, 1).

The results of the nitroblue tetrazolium test on the polymorphonuclear leukocytes from the pleural fluid were highly variable in all of the diagnostic categories (Fig 1). Patients with bacterial pneumonia could not be separated from those in the other diagnostic categories with the results of the nitroblue tetrazolium test. The polymorphonuclear leukocytes from the patients with congestive heart failure took up the dye, as manifested by an average score of about 100. The greatest variation in the results of the nitroblue tetrazolium test was in the category of malignant pleuritis, where the scores ranged from 0 to 300. All three patients with collagen vascular disease had very high counts for the pleural polymorphonuclear leukocytes on the nitroblue tetrazolium test.

Figure 1. Results of nitroblue tetrazolium (NBT) tests on polymorphonuclear (PMN) leukocytes from pleural fluid in patients with varying diagnoses.

Figure 2. Results of nitroblue tetrazolium (NBT) tests on small lymphocytes from pleural fluid in patients with varying diagnoses.
The scores for the small lymphocytes on the nitroblue tetrazolium test were in general much lower than were the scores for the polymorphonuclear leukocytes (Fig 2). Of the 51 patients who had lymphocytes in their pleural fluid, 34 (67 percent) had counts of less than 25 on the nitroblue tetrazolium test. The patients with tuberculous pleuritis could not be separated from those with pleural malignant neoplasms or other diagnosis with this test. All three patients with collagen vascular disease had very high results on the nitroblue tetrazolium test for the pleural lymphocytes, as they did for their polymorphonuclear leukocytes.

The results on the nitroblue tetrazolium test for the mononuclear cells other than the small lymphocytes were higher than those on the polymorphonuclear leukocytes (Fig 3). The mean score for this category of cells for all patients was 183, compared to the mean score of 156 for the polymorphonuclear leukocytes and 53 for the small lymphocytes. The results of the nitroblue tetrazolium test for the mononuclear cells did not separate the patients with either pneumonia or tuberculosis from those with malignant neoplasms or congestive heart failure. Noteworthy is the high mean score on the nitroblue tetrazolium test for these cells in patients with congestive heart failure. The patients with collagen vascular disease again had the highest results on the nitroblue tetrazolium test for mononuclear cells, as they did for their polymorphonuclear leukocytes and their small lymphocytes.

Since there was such a wide range in the counts on the nitroblue tetrazolium test for the patients with pneumonia and pleural effusion (parapneumonic effusion), the data were examined to see if there was any relationship between the results of the nitroblue tetrazolium test on the pleural fluid and other values for the pleural fluid. The score on the nitroblue tetrazolium test did not correlate with the pH, glucose level, lactic dehydrogenase level, or the results of culture of the pleural fluid.

For patients with malignant pleural effusions, an attempt was made to correlate the results of the nitroblue tetrazolium test with the cellular type of the tumor. Even though the number of individuals in each category was small, there was still much overlap from one category to another, suggesting that the nitroblue tetrazolium test on pleural fluid is not useful in separating different types of malignant pleural effusions. In the malignant pleural effusion, the scores on the nitroblue tetrazolium test were not correlated significantly with the glucose level, lactic dehydrogenase level, pH, or white blood cell count of the pleural fluid.

For the first ten patients studied, nitroblue tetrazolium tests were also performed on the peripheral blood. Since there was virtually no correlation between the results for the peripheral blood and the results for the pleural fluid, we ceased performing the nitroblue tetrazolium test on the peripheral blood.

**Discussion**

In contrast to the results on the joint fluid, on which the nitroblue tetrazolium test is useful diagnostically, the nitroblue tetrazolium test is not particularly useful on pleural fluid. The results of the nitroblue tetrazolium test on pleural fluid are similar to those on serum in that a large number of different diseases are associated with positive results, and there is much overlap between groups of patients with various diagnoses. This conclusion is valid for the results of the nitroblue tetrazolium test on the small lymphocytes or the other mono-

**Figure 3.** Results of nitroblue tetrazolium (NBT) tests on mononuclear cells other than small lymphocytes from pleural fluid in patients with varying diagnoses.
nuclear cells, as well as for the polymorphonuclear leukocytes.

The one instance in which the nitroblue tetrazolium test on pleural fluid may be useful diagnostically is with collagen vascular diseases. The average score on the nitroblue tetrazolium test (obtained by adding the scores for each of the cellular types and dividing the number of cellular types present) exceeded 250 for each of the three patients with collagen vascular disease (Fig 4). In contrast, only two of the other 59 patients had an average score that exceeded 250. Both of these patients had carcinoma, and in one, only mononuclear cells were present. Further studies are necessary to confirm these initial observations on patients with collagen vascular disease, since the number of patients is small. Interestingly, the scores on the nitroblue tetrazolium test on peripheral leukocytes of patients with systemic lupus erythematosus or rheumatoid arthritis are not increased.

When one is dealing with a patient with a parapneumonic effusion, it is important to identify those patients who will require tube thoracostomy as early as possible, since drainage becomes progressively more difficult the longer it is delayed. Although positive bacterial cultures, positive Gram stains of the pleural fluid, high white blood cell counts in the pleural fluid, a low glucose level in the pleural fluid, and a low pH in the pleural fluid have been used as indications for tube thoracostomy, none is ideal; however, the nitroblue tetrazolium test does not yield additional useful information for identifying those individuals who eventually will need tube thoracostomy.

Most previous studies of the nitroblue tetrazolium test have concentrated on the results for polymorphonuclear leukocytes. Studies by Wenger and Bole and by Hedley and Currie did analyze the nitroblue tetrazolium test on peripheral blood and found that a much higher percentage of mononuclear cells than polymorphonuclear leukocytes were positive for nitroblue tetrazolium. The present study demonstrates that small lymphocytes and the other mononuclear cells from pleural fluid also take up and reduce nitroblue tetrazolium. As with peripheral blood the mean score on the nitroblue tetrazolium test for the pleural mononuclear cells other than the small lymphocytes was higher than the mean score for the polymorphonuclear leukocytes. The mononuclear cells in the present study included mesothelial cells, macrophages, plasma cells, and malignant cells. With several patients, malignant-appearing fragments of tissue from the pleural fluid were very positive for nitroblue tetrazolium.

REFERENCES

7. Light RW, Macgregor MI, Luchsinger PC, Ball WC Jr. Pleural effusions: the diagnostic separation of transm...
Denton A. Cooley Cardiovascular Surgical Society

The Eighth Annual Scientific Meeting of the Denton A. Cooley Cardiovascular Surgery Society will be held August 18-23 at the Inter-Continental Rio Hotel, Rio de Janeiro, Brazil. For information, contact Dr. J. Ruiz, 1106 South Druid, Clearwater, Florida 33516.

First National Congress on Respiratory Diseases

The Indian Chest Society will sponsor the First National Congress on Respiratory Diseases at the Hotel President, Colaba, Bombay, India, September 10-12. For details, please write to Dr. P. G. Kamath, Department of Chest Medicine, C.V.T.C., KEM Hospital, Bombay 400 012, India.

Second World Congress on Cardiac Rehabilitation

The Second World Congress on Cardiac Rehabilitation will be held under the auspices of the Scientific Council on Cardiac Rehabilitation of the International Society and Federation of Cardiology. The congress will be held at the Jerusalem Hilton Hotel, Jerusalem, Israel, November 30-December 3. For information, contact Dr. J. J. Kellermann, Head, Cardiac Evaluation and Rehabilitation Institute, Chaim Sheba Medical Center, Tel Hashomer, Israel.