Serologic Diagnosis of Syphilis

The Use of Treponema Pallidum Immobilization and Immune Adherence Tests

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• **TPI tests were carried out on 4,060 sera. Among 3,934 patients with reactive STS and no history of syphilis, 2,148 or 54.6 per cent had negative reaction to TPI tests, 1,695 or 43.1 per cent had positive reaction and 91 or 2.3 per cent had doubtful reaction.**

Two hundred and ninety-two or 73.0 per cent of 400 pregnant women with reaction to STS in the absence of a history of syphilis showed negative results by TPI test, 103 or 25.8 per cent had positive results and five or 1.2 per cent had doubtful reaction.

Ninety-five or 75.4 per cent of 126 patients with a history of treated syphilis had positive reaction to TPI tests, 20 or 20.2 per cent had negative reaction and nine or 9.1 per cent had doubtful reaction.

TPI and TPIA tests were done on 143 sera carefully selected for the study. Among 102 sera subjected to the TPI test, 46 or 100 per cent of these positive were also positive by TPIA tests, while 52 or 94.5 per cent of 55 TPI-negative sera were also nonreactive by TPIA test. One serum gave doubtful TPI test reaction and positive TPIA test reaction.

STANDARD SEROLOGIC TESTS (STS) employing lipoidal or cardiolipin antigens have been used routinely in the serologic diagnosis of syphilis since 1906. Inasmuch as such antigens are nonspecific, many investigators recognize that biologic false positive reactions (BFP) occur in patients with deranged physiologic states or with diseases other than syphilis. They stimulate the production of a nonspecific antibody, reagin, as measured by antigens employed in serologic tests for syphilis. Biologic false positive reactions occur in so-called normal persons and are considered by certain investigators to be presumptive evidence of a collagen disease. Conversely, the disappearance of reagin during the tertiary and latent stages of syphilis results in a biologic false negative (BFN) reaction. Because syphilis simulates many diseases, an indefinite history renders it difficult to determine whether a patient has syphilis or is a BFP reactor, especially if one considers the variable period of latency occurring in syphilitic infection.

The development of the *Treponema pallidum* immobilization (TPI) test by Nelson and Mayer[8] in 1949, employing virulent, motile *T. pallidum* as the antigen, made available for the first time a specific diagnostic procedure for syphilis. They observed that 89 per cent of patients clinically and epidemiologically classified as BFP reactors had negative reaction to TPI tests. Conversely, patients with clinically recognized tertiary syphilis but negative reaction to STS, had positive reaction to TPI tests. Their results have been duplicated by many investigators.[4, 5, 9] On the basis of results obtained in an extensive investigation, Moore and co-workers[6] said that approximately 50 per cent of patients who have positive reaction to STS but do not have a history of syphilis, are BFP reactors.

Although the TPI test has been established as the most specific and sensitive test for syphilis yet developed,[11] carrying out the test in the laboratory is highly technical and complex, thus limiting its use to a few laboratories throughout the world. As a result a search is in progress for a simpler test utilizing killed *T. pallidum* for an antigen so that the procedure may be employed in the routine clinical laboratory. The immune adherence (TPIA) test developed by Nelson[7] in 1953 shows promise. It is based on the principle that virulent *T. pallidum*, sensitized in the presence of antisyphilitic serum, adheres to the surface of human red cells in the presence of complement.

This communication provides further evidence of the value of the TPI test based on results obtained on 4,060 sera. In addition, a comparison of the sensitivity and specificity of the TPIA and TPI tests is presented.

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MATERIALS AND METHODS

The TPI test was carried out on 4,060 sera according to the method of Nelson and Mayer[8] with modifications as described by Magnuson and Thompson[3] and Boak and Miller.[1] Specimens of blood were obtained in specially processed glassware from private physicians throughout California and from the Los Angeles city and county health departments. The sera were removed and stored at —20° C. prior to testing.

The TPIA test was performed on 143 sera as described by Nelson[7] with modifications recommended by Rein.[10] The antigen employed was a heat-killed, merthiolate-preserved specimen of *T. pallidum* which was stable for at least two months.

RESULTS

Treponema Pallidum Immobilization Test

TPI tests were carried out on 4,060 sera. Of the total, 3,934 were reactive by standard serologic test but were obtained from patients with no history of syphilis. The remaining 126 sera were from patients with a history of treated syphilis.

Among the 3,934 patients with reactive STS and no history of syphilis, 2,148 or 54.6 per cent showed negative results by TPI tests and were classified as BFP reactors. Sixteen hundred and ninety-five or 43.1 per cent had positive TPI tests and 91 or 2.3 per cent gave doubtful reactions (Table 1). Pregnancy and approximately 100 diseases have been implicated as important factors in bringing about the BFP reactions. In the present series the greatest number of BFP reactions occurred among pregnant women. Two hundred and ninety-two or 73.0 per cent of 400 sera from pregnant women gave negative reaction to TPI tests, 103 or 25.8 per cent positive reaction and 5 or 1.2 per cent doubtful reaction[2] (Table 2).

The most common diseases reported by the physician in which BFP reactions occurred were:

Arteriosclerosis	Periarteritis nodosa
Brucellosis	Rheumatic fever
Cirrhosis of liver	Rheumatoid arthritis
Coccidioidomycosis	Sarcoidosis
Diabetes mellitus	Subacute bacterial endocarditis
Lupus erythematosus	Tuberculosis
Malaria	
Malignancy	

In a study of 126 patients with a known history of treated syphilis, 95 or 75.4 per cent had positive reaction TPI tests, 20 or 15.8 per cent had negative reaction and 11 or 8.8 per cent had doubtful reaction. Of 99 patients who had a history of primary or secondary syphilis, 70 (70.7 per cent) had positive reaction to TPI tests, 20 (20.2 per cent) had negative reaction and 9 (9.1 per cent) had doubtful reaction. Six patients with congenital syphilis had positive TPI tests, while 19 or 90.5 per cent of 21 patients with negative reaction to STS but who had signs and symptoms referable to tertiary syphilis, had positive reaction to TPI tests. The two remaining patients showed doubtful reactions (see Table 3).

Treponema Pallidum Immune Adherence Test

TPI and TPIA tests were done on 143 sera carefully selected for the study. In a preliminary experiment conducted on 41 sera, perfect correlation was observed as between results by both tests—agreement on positive reaction in 21 sera and on negative in 20. In subsequent tests on 102 sera previously tested by the TPI procedure, 46 or 100 per cent of the TPI-positive sera showed positive reaction to TPIA tests, while 52 or 94.5 per cent of 55 TPI-negative sera were negative to TPIA test. The remaining specimen gave doubtful reaction by TPI test, positive reaction by TPIA.

Perfect correlation resulted when 26 "normal" persons with nonreactive STS and TPI tests were also TPIA-negative. Among 25 patients with a known history of primary or secondary syphilis, there was disagreement in only three cases. Inasmuch as the TPIA test was reactive in two of the three sera in which the TPI test was nonreactive, the sensitivity of the TPIA test would seem to be greater than that of the TPI test. In 25 cases in which the patient had no history of syphilis, had positive reaction to STS and negative TPI test results, there was only one case, that of a patient with an inguinal lymphadenopathy, in which the result of the TPIA test was positive, suggesting the possibility of primary syphilis. When 26 patients with no history of syphilis but with positive reaction to STS and to TPI tests were tested by the TPIA procedure, no disagreement was noted.

TABLE 1.—*TPI Tests on Sera from 3,934 Patients Without History of Syphilis but With Positive Reaction to STS.*

	No.	Per Cent
TPI Negative	2,148	54.6*
TPI Positive	1,695	43.1
TPI Doubtful	91	2.3
Total	3,934	100.0

*Biologic False Positive Reactors.

TABLE 2.—*Results of TPI Tests on 400 Sera from Pregnant Women With Positive Reaction to STS.*

TPI	Positive STS	Per Cent
Negative	292	73.0*
Positive	103	25.8
Doubtful	5	1.2
Total	400	100.0

*Represents Biologic False Positive Reactors.

DISCUSSION

The TPI test is extremely valuable in differentiating BFP reactions from those due to infection with *T. pallidum*. The percentage of BFP reactors, as measured by the TPI test, has increased each year, indicating the important role of the TPI test as an aid in more accurate differential diagnosis. The trend may be due to an increased sensitivity of the

TABLE 3.—*TPI Tests on Sera from 126 Patients With History of Syphilis.*

Classification Treated Syphilis	Results of TPI Tests						
	Positive	Per Cent	Negative	Per Cent	Doubtful	Per Cent	Total
Primary and secondary	70	70.7	20	20.2	9	9.1	99
Tertiary (clinical) negative STS	19	90.5	0	0	2	9.5	21
Congenital	6	100.0	0	0	0	0	6
Total	95	75.4	20	15.8	11	8.8	126

various lipoidal or cardiolipin antigens, to variation in technical performance of the tests in different laboratories, or to an increase in certain degenerative diseases or abnormal physiologic states occurring among the population.

Studies designed to investigate the sensitivity and specificity of the TPI test rely to a large extent on obtaining dependable histories of syphilis and therapy from the patient and physician. Such information is often difficult to get and its accuracy must be considered in any evaluation of the TPI test. Although the 20 negative results by TPI test in patients with a history of primary or secondary syphilis may be explained on the basis of adequate therapy prior to the appearance of immobilizing antibodies, the foregoing possibilities must be considered.

The TPIA test, on the basis of results obtained to date, offers promise as a supplement to the TPI test. Inasmuch as a killed treponemal antigen may be employed, it can be more readily adapted for use in a routine clinical laboratory. Further studies concerning this test are in progress.

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REFERENCES

1. Boak, R. A., and Miller, J. N.: A simple medium for maintaining the viability of *Treponema pallidum* in the *Treponema pallidum* immobilization test, Am. J. Syph., Gonor. and Ven. Dis., 38:429, 1954.

2. Boak, R. A., Carpenter, C. M., Miller, J. N., Drusch, H. E., Chapman J. M., and Heidbreder, G. A.: Pregnancy: A factor responsible for biologic false positive reactors as determined by the *Treponema pallidum* immobilization test, Surg., Gyn. & Obst., 101:751, 1955.

3. Magnuson, H. J., and Thompson, F. A.: Treponemal immobilization test of normal and syphilitic serums, J. Ven. Dis. Inform., 30:309, 1949.

4. Miller, J. L., Slatkin, M. H., Lupton, E. S., and Brodey, M.: Studies on the value of the TPI test in the diagnosis of syphilis, Am. J. of Syph., Gonor. and Ven. Dis., 36:559, 1952.

5. Mohr, C. F., Moore, J. E., Nelson, R. A., and Hill, J. H.: Studies on the relationship of treponemal antibody to probable biologic false serologic tests for syphilis, Am. J. Syph., Gonor., and Ven. Dis., 34:405, 1950.

6. Moore, J. E., and Mohr, C. F.: The classification, recognition, incidence, and etiologic background of false positive serologic tests for syphilis, J.A.M.A., 150:467, 1950.

7. Nelson, R. A.: The immune adherence phenomenon. An immunologically specific reaction between microorganisms and erythrocytes leading to enhanced phagocytosis, Science, 118:733, 1953.

8. Nelson, R. A., and Mayer, M. D.: Immobilization of *Treponema pallidum in vitro*, by antibody produced in syphilitic infection, J. Exper. Med., 89:369, 1949.

9. Nelson, R. A., Zheutlin, H. E., Diesendruck, J. A., and Austin, P. G.: Studies on treponemal immobilizing antibodies in syphilis. II. Incidence in serum and cerebrospinal fluid in human beings and absence in biologic false positive reactors, Am. J. Syph., Gonor. and Ven. Dis., 34:101, 1950.

10. Rein, C.: Personal communication.

11. Zellman, H. E.: Specificity of the treponemal immobilization test, Am. J. Syph., Gonor. and Ven. Dis., 38:506, 1954.