# The False Positive Reactions for Syphilis as a Problem in Medical Practice

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**Abstract**

This paper presents the primary causes of false positive reactions for syphilis and describes clinical cases of patients with false-positive serological reactions. The data suggest that positive reactions to either the nontreponemal or the treponemal test do not always indicate the presence of syphilis.

**Keywords**: syphilis, false positive serologic reactions to syphilis, immunoblotting.

**Ложноположительные реакции на сифилис – проблема в медицинской практике**

В работе рассмотрены основные причины ложноположительных реакций на сифилис (ЛППРС) и представлено описание клинических случаев острых ЛППРС у пациентов с различными заболеваниями. На основании собственных данных высказывается мнение, что положительные трепонемные и нетрепонемные реакции не всегда свидетельствуют о наличии сифилиса.

**Ключевые слова**: сифилис, ложноположительные реакции на сифилис, иммуноблотинг.

In the Republic of Moldova, syphilis is still a great public health problem. The World Health Organization estimates that there are approximately 12 million new cases of syphilis in adults worldwide. The vast majority of these are seen in developing nations, but an increase in new cases has also been noted in Eastern Europe since the dissolution of the Soviet Union.

Laboratorial diagnosis of syphilis is crucial to the epidemiological and diagnostic evaluation of the disease. Syphilis has several clinical manifestations, making laboratory testing a very important aspect of diagnosis. Despite several advances in key areas, the management of patients with syphilis remains difficult and controversial (8, 10). In the Republic of Moldova, many unsuspected cases are discovered by laboratory testing.

The serologic detection of specific antibodies to T. pallidum is of particular importance in the diagnosis of syphilis, since the natural course of the infection is characterized by periods of latency. Latent syphilis can only be diagnosed by serological tests. In fact, in the Republic of Moldova the majority of syphilis cases are identified at the latent stage by serological tests (55%). The etiological agent, Treponema

pallidum, cannot be cultured, and there is no single optimal alternative test.

The complexity of syphilis serology means that the services of reference laboratories and clinical experts are often needed. Therefore, the laboratorial diagnosis is performed mainly by serological tests, which are divided into nontreponemal tests for screening and treponemal tests for confirmation. The nontreponemal tests include the Venereal Disease Research Laboratory (VDRL), RPR (Rapid plasma reagin) which is useful for screening and evaluation of treatment responses.

Treponemal tests are used to confirm the diagnosis and include hemagglutination assay (TPHA), fluorescent treponemal antibody absorption (FTA-ABS) as well as enzyme-linked immunosorbent assay (ELISA), and to detect specific anti-treponemal antibodies. All nontreponemal tests measure both immunoglobulin IgG and IgM antiphospholipid antibodies, formed by the host in response to lipoidal material released by damaged host cells early in infection, and lipids from the cell surfaces of the treponem itself. All treponemal tests use Tr. pallidum or its components as the antigen. If lesion exudate or tissue is available, direct examination is performed, followed by a nontreponemal serology test. A reactive nontreponemal test is then confirmed by a treponemal test. A confirmed serological test result is indicative of the presence of treponemal antibodies but does not indicate the stage of disease, and depending on the test, it may not differentiate between past and current infection. The sensitivity and specificity of serological tests vary depending on the type of test and stage of the disease.

All of the serologic tests for syphilis have been shown to possibly give false results when several different conditions are present: other spirochetal diseases, autoimmune disorders or human immunodeficiency virus infection. Consequently, the use of a single method is considered insufficient to achieve the best diagnostic performance, and the quest for new, simple, reliable and money-saving diagnostic methods continues. In theory, a confirmatory serologic test should have at least equivalent sensitivity, but greater specificity, than the screening test that uses a different methodology (4, 11).

Nontreponemal tests are widely available, rapid, inexpensive and are necessary for determining the efficacy of treatment or confirming the reinfection. There are, however, a number of limitations associated with nontreponemal tests:

- First, they lack sensitivity in late stage of infection: 30% of patients with late latent or late active syphilis will show a non-reactive result.
- Secondly, 1-2% of patients with secondary syphilis exhibit a prozone reaction. Prozone occurs when an excess of antibody in undiluted serum inhibits flocculation with the antigen, giving rise to weakly reactive, atypical or occasionally false negative results.
- Finally, antibodies detected by nontreponemal tests are not only produced as a consequence of treponemal infection, but also in response to other conditions where tissue damage occurs.

Because nonspecific or false-positive reactions occur with the nontreponemal tests, usually as a result of damage to the host's tissue by infection, immunization or autoimmune disease, the search for a specific serologic test for syphilis continues. The incidence of false-positive reactions depends on the test used and the population studied (1, 4). Therefore, false-positive nontreponemal test reactions can have multiple causes, their incidence is generally 1% to 3% (6, 8). The rate of false-positive tests during pregnancy is greater than that seen in the general population. Additionally, as many as 28% of positive RPR results in pregnant women are biological false positives (13). It is also about 10% higher among intravenous drug users. The interpretation of the results of the nontreponemal tests depends on the population being tested. The predictive value of the nontreponemal tests is increased when combined with a reactive treponemal test. Therefore, when the nontreponemal tests are used as screening tests in a low-risk population, all reactive results should be confirmed with a treponemal test. In some low-risk populations, every reactive result may be a false-positive result (12).

False-positive reactions occurring with the nontreponemal tests can be divided into two groups: those that are acute false-positive reactions of <6 months in duration and those that are chronic false-positive reactions that persist for >6 months (8, 9). Acute false-positive nontreponemal reactions have been associated with hepatitis, infectious mononucleosis, viral pneumonia, chicken pox, measles, other viral infections, malaria, immunizations, pregnancy, tuberculosis, Lyme disease, neoplasm, HIV infection and laboratory or technical error (4, 5, 8).

Chronic false-positive reactions have been associated with connective tissue diseases, such as systemic lupus erythematosus or diseases associated with immunoglobulin abnormalities, which are more common in women; thus, chronic false-positive reactions are more common in women than in men. Other conditions associated with chronic false-positive reactions are narcotic addiction, aging, leprosy and malignancy. Generally, up to 90% of false-positive reactions have a titer of less than 1:8, and reactive nontreponemal tests with titers less than 1:8 and subsequent nonreactive treponemal tests are considered to be biological false-positive reactions.

The titer of false-positive reactions is usually low, but on rare occasions it can be extremely high; therefore, the quantitative titer cannot be used to differentiate between a false-positive reaction and syphilis. This is especially true for persons who inject illegal drugs. More than 10% of intravenous drug users have false positive test results with titers less than 1:8. Chronic false-positive reactions persist for more than six months and are often associated with autoimmune disorders and chronic inflammatory conditions (4, 6, 8, 11).

The problem is complicated because false-positive reactions can also occur with treponemal tests.

The specificity of the FTA-abs (fluorescent treponemal antibody) is also poorer than that of the other treponemal tests.

Approximately 1-2% of the normal population will show a false positive FTA-abs result. False positives have also been

reported with narcotic addiction, autoimmune haemolytic anaemia, some viral infections and in the elderly (5).

The TPHA test is highly sensitive in all stages of the disease except, possibly, in early primary syphilis. It also has very high specificity with as few as 1.5% false positives. These false positives have been reported in some patients with infectious mononucleosis, connective tissue diseases, leprosy and with intravenous drug use. The EIA can be false positive in LES, neoplasmes, leprosy, rheumatoid arthritis. The non-recognition of serological false-positive tests for syphilis may have negative prognostic and social implications. Therefore, careful clinical interpretation of test results and other evidence is necessary for proper diagnosis. Significant research advances in recent years have influenced the management of patients with syphilis.

The ideal test for syphilis should have both high sensitivity and specificity, be suitable for monitoring response to treatment, give a negative result after adequate therapy and also give a clear indication of reinfection. Unfortunately, such a test does not exist. Instead a combination of tests must be used (14). Technological advances have resulted in improved serodiagnostic tools for syphilis.

The Western blot (WB) method has been used for the past 15 years to investigate the immune response to individual Treponema pallidum antigens in sera from experimentally infected animals and from humans with naturally occurring syphilis.

Immunoblotting allows for the detection of antibodies to individual proteins. In the Treponemal Western blot, solubilized T.pallidum proteins are separated by gel-electrophoresis according to their molecular size. The separated proteins are then transferred onto a nitrocellulose membrane which is dried and cut into strips. After incubating the strips with patient's serum, antigen-antibody complexes are visualized by adding enzyme-conjugated anti-human globulin followed by substrate, which causes a color reaction. It is generally agreed that detection of antibodies to immunodeterminants with molecular masses of 15, 17, 44.5 and 47kDa are diagnostic for acquired syphilis (3, 9, 14). A recent development is the use of recombinant antigens instead of fractionated proteins. The use of recombinant antigens could avoid the difficulties in purifying specific T. pallidum proteins due to the complex antigenic structure of this spirochete, and it has the potential to increase the specificity of serologic investigations (3, 7, 9).

Currently the Treponemal Western Blot Test is a confirmation test for syphilis. It is not intended to be used for routine confirmation, but is reserved for situations where the clinical picture and other serological test results do not give a clear status of infection. The western blot has highest specificity and sensibility in all stage of syphilis (2, 7, 9). The IgG immunoblot using recombinant antigen is recommended as supplementary confirmatory test when a positive EIA screening test is not confirmed by the TPPA (TPHA) test or when a positive TPPA (TPHA) screening test is not confirmed by the EIA test (2).

Finally, we would like to present our cases of personal medical practice.

Patient N., 71 years old, is suffering from hepatitis C, hepatitis B and rheumatoid arthritis. He had no evidence of clinical symptoms or history of syphilis. His wife's serological studies for syphilis were negative. He had the RMP 1:8 titer, EIA weakly reactive, TPHA 1:80,WB negative. We diagnosed the false-positive test for syphilis in this patient.

Patient K., 20 years old, virgin, is suffering from rheumatism. He had no evidence of clinical symptoms or history of syphilis. He had RW 4+ 1: 5 titer. RMP1:3 titer, TPHA 1:80, WB negative, ELISA negative. We diagnosed the false-positive test for syphilis in this patient.

Patient W., 53 years old, is suffering from prostate cancer with metastasis. His wife's serological studies for syphilis were negative. He had no evidence of clinical symptoms or history of syphilis. RMP 4+ 1:3 titer, RW 4+ 1:10, TPHA and EIA weakly positive. We diagnosed the false-positive test for syphilis in this patient.

Patient R., 24 years old, is pregnant, suffering from hepatitis B and genital herpes. Her husband's serological studies for syphilis were negative. He had no evidence of clinical symptoms or history of syphilis. Serological tests: RW3+, RMP3+, TPHA negative, EIA reactive, WB negative. We diagnosed the false-positive test for syphilis in this patient.

Patient Z., 49 years old, suffering from diabetes, chronic renal insufficiency and is on hemodialysis. He had no evidence of clinical symptoms or history of syphilis. His wife's serological studies for syphilis were negative. Serological results: RW4+ 1:10, RMP 4+1:4, TPHA, EIA reactive, WB negative. We diagnosed the false-positive test for syphilis in this patient.

Patient X., 35 years old, is suffering from Lyme disease. He had no evidence of clinical symptoms or history of syphilis. His sexual partner's serological studies for syphilis were negative. Serological tests results: RW 4+1:10, RMP4+1:4, EIA, TPHA reactive, WB negative, Lyme test titer positive. We diagnosed the false-positive test for syphilis in this patient.

In conclusion, serologic tests provide only indirect evidence of syphilis and may be reactive in the absence of clinical, historical or epidemiologic evidence of syphilis, and are, therefore, very important for the laboratory diagnosis to be as reliable as possible. We can conclude that many of the false-positive reactions can be resolved using the Western-blot assay and its use can improve the reliability of syphilis serology.

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