THE DIAGNOSTIC PERFORMANCE OF TREPONEMAL WESTERN BLOT AS A CONFIRMATORY TEST FOR LATENT SYPHILIS

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Резюме

Определение диагностических возможностей реакции иммуноблот (вестерн блот) при раннем скрытом сифилисе и ложноположительных реакциях на сифилис

Статья посвящена вопросам дифференциальной диагностики раннего скрытого сифилиса и ложноположительных реакций на сифилис. Серологические реакции играют значительную роль в диагностике сифилиса и часто являются единственным практическим диагностическим критерием. Данный вопрос особенно актуален в период высокой заболеваемости сифилисом в Республике Молдова, сопровождающейся возрастанием удельного веса скрытого сифилиса. В клинической практике встречаются ситуации, когда серологическое тестирование дает отрицательные результаты, нетрепонемные тесты могут быть ложно отрицательными у пациентов со злокачественным сифилисом, первичным или приобретенным иммунодефицитом. Трепонемные тесты более чувствительны, но не абсолютно специфичны и могут быть ложно положительными при колланенозах, аутоиммунных заболеваниях, некоторых инфекциях, беременности, онкологических заболеваниях и др.

Проведенные нами исследования показывают, что на данный момент реакция иммуноблота (иммуноблоттинг) является наиболее чувствительным и специфичным тестом при сифилисе и используется в случаях, когда другие специфические тесты (реакция прямой гемагглютинации, иммуноферментный анализ) дают неопределенный ответ или ложноположительный результат.

Ключевые слова: *ранний скрытый сифилис, ложноположительный результат, реакция иммуноблот.*

Rezumat

Evaluarea posibilităţilor de diagnostic prin reacţia immunoblot în sifilisul latent şi în reacţiile serologice fals pozitive

Articolul abordează aspectele practice ale diagnosticului diferenţiat al sifilisului latent recent şi al reacţiilor serologice fals pozitive la sifilis. În R. Moldova, în structura morbidităţii înalte prin sifilis se constată o pondere stabilă a sifilisului latent recent (circa 50-55%). Pentru menţinerea controlului asupra morbiditaţii prin sifilis este necesar un diagnostic precis şi rapid al acestei maladii, dar în pofida succeselor diagnosticului de laborator, managementul pacienţilor cu sifilis latent rămâne dificil şi controversat. Există situaţii în care testarea serologică furnizează rezultate negative: testele netreponemice în sifilisul latent tardiv şi în cel terţiar sunt fals negative în 30-33% din cazuri, reacţiile serologice cu teste netreponemice pot fi fals negative la pacienţii cu sifilis malign, imunodeficienţe primitive sau secundare (SIDA etc.). Testele serologice treponemice sunt mai sensibile, dar nu au o specificitate absolută (pot fi fals pozitive în colagenoze, maladii autoimune, unele infecţii, în sarcină, tumori), iar dacă sunt efectuate persoanelor cu risc scăzut de sifilis (screening), pot da un procent ridicat de RSFP (testul FTAabs la 1% din populaţia generală).

Rezultatele studiului nostru au demonstrat că reacţia immunoblot (western blot) este cel mai sensibil şi specific test şi poate fi utilizat în situaţiile în care alte reacţii specifice dau un rezultat neclar, echivoc sau fals pozitiv.

Cuvinte-cheie: *sifilis latent, reacţii serologice fals pozitive, reacţia immunoblot.*

Introduction

Syphilis is a chronic bacterial infection that remains a public health concern worldwide. Although syphilis rates are quite low in industrialized countries, the World Health Organization estimated that 12 million new cases of venereal syphilis occurred in 2001 [12], more than 90% of them in developing countries, with a rapidly increasing number of cases in Eastern Europe.

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 latent syphilis remains difficult and controversial [8, 10].

The problem acquires special importance in the conditions of the high syphilis morbidity in the Republic of Moldova, associated with an enhanced percentage of latent syphilis (50%). Serological tests for syphilis continue to play a major role in the diagnosis and management of the disease and often are the only practical means of diagnosis .

The antibodies detected by non-treponemal tests are not only produced as a consequence of treponemal infection, but also in response to other condition were tissue damage occurs. 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, malignancy, or human immunodeficiency virus infection (3, 4, 6). Consequently, the use of a single method is considered insufficient to

achieve the best diagnostic performance; both non-treponemal and treponemal serological tests should be carried out in all clinically suspected cases.

The sensitivity and specificity of serological tests vary depending on the type of test and stage of the disease [6].

The main limitations of non-treponemal tests are their reduced sensitivity in primary syphilis and late latent syphilis (30% false negative), false-positive results due to crossreactivity, and the potential for false-negative results due to prozone reactions (1-2%). Prozone reactions are false-negative reactions that occur due to interference by high concentrations of target antibodies in a specimen.

Treponemal tests are used mainly as confirmatory tests to verify reactivity in non-treponemal tests. Treponemal tests are also used as diagnostic tests in patients with nonreactive non-treponemal tests but with signs and symptoms of late syphilis.

The phospholipid antibodies detected by non-treponemal tests are not only produced in syphilis and other treponemal disease but also in response to a variety of conditions unrelated to syphilis. Therefore, false-positive non-treponemal test reactions can have multiple causes. Their incidence is generally 1% to 2% [4, 11]. False reactive results may be more frequent when testing certain patients groups, such as the elderly or the pregnant, or patients with drug addiction, malignancy, autoimmune diseases (for example, systemic lupus erythematosus), viral diseases (particularly with Epstein–Barr and hepatitis viruses), protozoal, or mycoplasma infection [1, 2, 5, 7]. In low risk populations, all reactive test results should be confirmed by a treponemal test since over 50% of the non-treponemal tests may be false reactive.

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. It is generally agreed that detection of antibodies to immunodeterminants with masses of 15, 17, 44.5 and 47 kDa are diagnostic for acquired syphilis. The western blot has highest specificity and sensibility in all stage of syphilis [5, 7, 9, 13]. 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 .

The goal of study was the evaluation the Western blot assay in order to determine the sensitivity and specificity of the test and compare the results with those of other confirmatory assays.

Materials and methods

Sera were obtained from 4 different groups of subjects: I group of 107 specimens was obtained from patients with early latent syphilis; the II source of sera was a group of 81 patients who were suffering from primary (21) and secondary (60) syphilis; the III group of 81 serum samples was obtained from patients who showed clinical and laboratory conditions well known to be potential causes of false-positive reactions in the serologic diagnosis of syphilis; the IV group of 128 serum samples obtained from dermatological patients.

All sera were tested by RMP (reaction of microprecipitation), RW (Wasserman test), TPHA (*T. pallidum* hemagglutination assay), ELISA (enzyme linked immunosorbent assay), WB (Western blot, EUROIMMUN AG, Germany). The tests were carried out according to the manufacturer's instructions.

Consensus results by classical tests

For the 397 well documented samples, we established a consensus serological diagnostic for all samples on the basis of the available results of the classical assays (TPHA, ELISA). In some cases, if enough serum was available, repeat testing. In some cases, the global information was considered to define the consensus results. Since RMP is often negative for patients with true late infections (who are positive by other techniques), RMP data were not used to determine the consensus results. These consensus results were obtained as follows. A sample was considered positive if all available results were positive; a sample was considered negative if all available results were negative. When discrepant results were shown, the most predominant result was considered the consensus result; if discrepant results were present in equal numbers, no consensus could be reached and the result for the sample was therefore considered to be equivocal.

Table 1

Sensitivity of serologic tests for syphilis according to the stage of disease

	RMP	*RW*	*ELISA*	*TPHA*	*WESTERN BLOT*
PRIMARY SIFILIS (21 PACIENTS)	100%	100%	100%	90%	100%
SECONDARY SIFILIS (60 PACIENTS)	100%	100%	100%	100%	100%

Note. All the patients with primary syphilis had non-treponemal tests (RMP, RW) positive.

All 81 sera samples from clinical phases of syphilis were positive by WB-IgG, giving a sensibility of 100%.

The results also showed that the concordance between WB and RMP, RW, ELISA were 100%, the agreement between the WB method and TPHA was 90% (test showed lower reactivity in the primary syphilis).

Table 2

Serological findings in patients with latent syphilis

TESTS	HIGH POSITIVITY	POSITIVITY	LOW POSITIVITY	NEGATIV RESULT	TOTAL POSITIVITY
MRP	50 (46,7%)	41 (38,3%)	6 (5,6%)	2 (1,8%)	**92,53%**
RW	51 (47,6%)	44 (41,1%)	5 (4,6%)	1 (0,9%)	**94,4%**
TPHA	87 (81,3%)	13 (12,1%)	3 (2,8%)	0	**97,28%**
ELISA	88 (82,2%)	11 (10,3%)	4 (3,6%)	0	**96,27%**
IMMUNOBLOT	99 (92,5%)	8 (7,5%)	0	0	**100%**

When used to define the immune response to *T. pallidum* antigens in sera obtained from patients with latent syphilis WB showed a good diagnostic performance when compared sensitivity to RMP (92,5%), RW (94,4%), ELISA (96.2%), TPHA (97,2%). The Western blot assay had the highest number of reactions, demonstrated 100% sensitivity.

Table 3

Treponema Pallidum Western blot antigens recognized by IgG antibodies in sera obtained from patients suffering from syphilis

Trep.Pallidum antigen	*Primary syphilis N=21*	*Secondary syphilis n=60*	*Latent syphilis n=107*
15 kDa	18 (85,7%)	51 (85,0%)	85 (79,4%)
17 kDa	17 (80,9%)	54 (90,0%)	86 (80,3%)
45 kDa TmpA	20 (95,2%)	60 (100%)	100 (93,4%)
47 kDa	19 (90,47%)	60 (100%)	98 (91,5%)
Sumar positivity	100%	100%	100%

The most frequently reactive among the antigens in WB was TmpA, 94,7% of the specimens being reactive. Two lipoproteins with molecular weights of 44.5 kDa and 47 kDa showed stronger antigenicity. The percentages of specimens reactive with *T. pallidum* antigens, other than TmpA, were as follows: 47 kDA – 94,1%; 17 kDa – 83,5%, and 15 kDa – 81,9%. The antigenic profile in latent and manifest (primary, secondary syphilis) it is very similar. In secondary and in early latent syphilis, antibodies reacted with high numbers of antigenic proteins of *T. pallidum.*

We tested 128 sera samples from blood of dermatological patients groups to analyze the specificity of immunoblot test.

Table 4

Results of reactive non-treponemal and treponemal tests with serum samples from dermatological patients group

N. OF SAMPLES	RW	RMP	ELISA	TPHA	WESTERN BLOT
4	R	R	N	N	N
12	R	R	N	N	N
19	R	N	P	N	N
21	R	N	N	N	N
86	R	R	P	N	N
94	N	N	N	P	N
106	R	R	N	N	N

Note: R – reactive, N – nonreactive

The specificity (Sp) of the treponemal tests based on the number of sera samples that reacted in isolated tests showed greater for WB-IgG, Sp = 100% (128/128); followed by TPHA, Sp = 99.2% (127/128); and ELISA Sp = 98,.4% (126/128).

TPHA was reactive for the serum sample from a patient with hepatitis. ELISA was reactive for sera samples from 2 patients, of whom one had toxoplasmosis, considered a false-positive reaction, and the other, hepatitis.

The rates of false-positivity of RMP and RW were higher than in treponemal tests. Numerous conditions have been associated with false-positive non-treponemal test results including other infections, pregnancy, connective tissue diseases, autoimmune disease malignancy, and narcotic addiction.

The WB gave the lowest overall percentage of false-positive reactions (1.08%), showed a 98,92% of specificity in this group of sera, followed by TPHA (95,6%) and ELISA (93,4%).

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. RMP 1:3 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 stu-

dies for syphilis were negative. He had no evidence of clinical symptoms or history of syphilis. Serological tests: RW 3+, RMP 3+, 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: RW 4+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, RMP 4+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. The non-recognition of serological false-positive tests for syphilis may have negative prognostic and social implications. The findings of our study demonstrate the high sensitivity and specificity of the Treponema pallidum Western blot assay, together with its simplicity and objectivity, make it a good confirmatory test for syphilis. 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.

Table 5

Results of the different serological tests in patients with biological false positive syphilis serology

Serological test	*RMP*	*RW*	*TPHA*	*ELISA*	*Immunoblot*
Low positivity	26 (28,2%)	34 (36,9%)	1 (1,08%)	4 (4,32%)	1 (1,08%)
Positive	51(55,4%)	46 (50,0%)	2 (2,17%)	2 (2,17%)	0
High positivity	14 (15,2%)	10 (10,8%)	1 (1,08%)	2 (2,17%)	0
Negative	1 (1,08%)	2 (2,17%)	88 (95,6%)	86 (93,4%)	91 (98,9%)

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