Serological diagnosis of syphilis*
*Diagnóstico sorológico da sífilis**

Osmar Rotta[1]

Eighty years ago, Arlindo de Assis, an assistant of the *Instituto Vital Brasil* published a long article entitled "*O immunodiagnostico da syphilis na actualidade*" (The current immune diagnosis of syphilis), in which he reported and discussed about several serological techniques that were under development in the world in the distant year of 1925. Since it is not our purpose to summarize the full text, we cite some parts of the article showing the author's concerns.[1]

> ..."The experience progressively taught us that the serodiagnosis of syphilis is based on abnormal reactions of organic liquids which are modified by the disease, over colloidal suspensions of certain lipids. There are strong reasons to suspect that such reactions have specific nature; the unexpected aspect of the phenomenon and the unique complexity of its individualization created, however, in the beginning, severe and compromising misunderstandings; however, the continued improvement of the technique and of interpretations have corrected them and gradually headed towards their elimination."
>
> ..."Actually, the contact of the colloidal system present in the fluids of healthy individuals or, in general, not suffering from syphilis, is not accompanied by any significant consequence; the colloidal mixture of these lipids with normal liquids indistinctly continues to flocculate at a slow pace, like any other similar systems.
>
> Syphilis, nevertheless, changes the interior medium in such a manner that, from then on, the contact of fluids with lipids triggers a huge unbalance between the two existing colloidal systems, thus making flocculation significantly faster."
>
> ..."In general practice, the [methods] most often used are the extracts of ox heart, particularly when carefully added with cholesterin, as taught by Sachs, in 1917. These are followed by human heart, syphilitic liver (the so-called abundance of treponemas in the organs seems to be not important at all), and guinea pig heart."
>
> ..."However, we do not intend to deny that Wassermann's reaction, or its variants, may be positive in the absence of syphilis and in other diseases. But these conditions are much less frequent than what is stated, and their diagnosis is relatively easy [to make]: treponemotasis (yaws) and some cases of tuberous leprosy."
>
> ..."In other infectious diseases (paludism, tuberculosis, gonorrhea, pneumococcal infections), in some constitutional affections (diabetes), in transient intoxication states (alcoholism, narcosis) and even in certain physiologic conditions (pregnancy, digestive period), some false reactions have been observed."
>
> ..."III - *Flocculation reactions* - The flocculation processes could not replace Wassermann's reaction yet. However, there were many cases of syphilis with a negative reaction and positive flocculation processes. Therefore, it is recommended to associate both reactions."
>
> ..."Hence, everything tends to prove that, if the mechanism of fluid changes in syphilis made general and speculative progresses, in practice, the most loyal demonstration of such changes is the hemolysis test, discovered by Wassermann. These tests would be even better if they could appropriately combine with a uniform technique. Flocculation and turbidity should become practice, since it is the path to improvement; they correspond to facts of safe observation and probably correct interpretation. However, at this moment, it would be more prudent to consider Wassermann's reaction as the basis for the immune diagnosis of lues."

Eighty years later, the serological reactions with lipid antigens are still useful and important for diagnosis and control of cure. Throughout those years, the treponemic tests were added to the diagnostic armamentarium of syphilis, together with, in the past decade, the nucleotide amplification methods.

Received on April 18, 2005.
Approved by the Editorial Council and accepted for publication on May 09, 2005.
* Work done at Escola Paulista de Medicina, São Paulo (SP) - Brazil.

[1] Professor of the Department of Dermatology of Escola Paulista de Medicina da Universidade Federal de São Paulo (SP).

LIPID OR REAGINIC TESTS

These tests detect IgG and IgM antibodies against serum lipids due to damage to the mitochondrial membrane of the host, and against lipids in the membrane of *Treponema pallidum*. The flocculation reactions - particularly VDRL - replaced complement fixation reactions, which were first described by Wassermann, Neisser & Bruck, in 1906. Even among several other flocculation reactions reported (Hinton, Kline, Khan, Mazzini, Meinicke), the VDRL remains predominant,[2] and is recommended by the World Health Organization. Its antigen is uniform in all laboratories since 1941, when Pangborn isolated the active ingredients of the extract of ox heart used at that time. The major setback is the need to prepare the mixture of cardiolipin (0.03%), cholesterol (0.9%) and lecitin (0.21%) every day.

The VDRL test becomes positive five to six weeks after infection; therefore, it may be negative in case of short-duration chancre. It is highly sensitive* in secondary syphilis (100%), and sensibility drops to 70% in late forms. The rare false-negative cases result from excess antibodies that induce a technical failure known as prozone phenomenon, which is more marked in cases of syphilis associated with Aids.

It is highly specific (99-100%) in the healthy population, and there are false-positive reactions in several morbid conditions (Chart 1).

It is useful to control cure and the serological follow-up after treatment should be performed every three months in the first year, and every month in the second year, when the test will be negative or there will be persistent low titers (serological scar).[3] Serological scar is considered the persistence of reagins in low titers (of pure serum up to 1:4) after two years, with positive treponemic tests. Persistent high titer serology, even with normal CSF, should be followed up longer due to possible existence of other treponema reservoirs. In asymptomatic cases, retreating is not effective.

The fast macroscopic reading tests play an important role in population detection and in clinics for asymptomatic homosexual and bisexual individua.[4] The rapid plasma reagin test (RPR) uses charcoal particles as an indicator; the screening test (RST) uses Sudan Black B, a liposoluble dye; and the toluidine red unheated serum test (TRUST) uses an azo pigment. The future trend of these fast tests is to be replaced by treponema antigens.

TREPONEMIC TESTS

The major difficulty in a developing a better use of *Treponema pallidum* as an antigen is the absence of *in vitro* culture. In 1949, Nelson and Meyer developed the immobilization test (TPI), which became the gold standard to diagnose syphilis. Its expensive and demanding performance restricted its use for academic purposes. To obtain live treponemas, it is necessary to make several inoculations in rabbit testes (Nichols strains, 1917), which will take from 11 to 28

CHART 1: Main causes of false-positive reactions

LIPID	Infections/ infestations	malaria	bacterial endocarditis
		Hansen's disease	measles
		typhus	varicella
		viral pneumonial	filariasis
		mononucleosis	trypanosomiasis
		tuberculosis	leptospirosis
		hepatitis	
	Immune diseases	systemic lupus	
		polyarteritis nodosa	
		rheumatoid arthritis	
	Other conditions	pregnancy	
		illicit drugs	
		senescence	
TREPONEMIC		collagen diseases	
		healthy individuals with rheumatoid factor	
		Hansen's disease patients	

* By and large, the term sensibility is used as the capacity to detect real cases of the disease, whereas specificity means the capacity to not detect healthy people as ill individuals.

CHART 2: Diagnostic resources in different stages of syphilis

SERONEGATIVE PRIMARY
- **Dark ground microscopy examination for Tp**
- **Chancre smear PCR**

SEROPOSITIVE PRIMARY/SECODARY
- **VDRL plus HA-Tp or FTA-abs**

LATENT
- **VDRL plus HA-Tp or FTA-abs**
- **CSF**

LATE
- **VDRL plus HA-Tp or FTA-abs**

+
Histopathology
+
Tissue PCR
+
CSF

days to develop orchitis. The live treponemas obtained are then immobilized by the presence of serum antibodies: if 50% or more are immobilized, the result is positive[5] (disease), if less than 20%, it is negative. The test is highly sensitive and specific (99%), and the presence of antibiotics in serum leads to false-negative results. The practical difficulties led to developing the complement fixation reaction with dead treponemas (Reiter), which is highly unspecific. The following reactions were specific: hemagglutination (Rathlev, 1967)[6] and FTA-abs. The tests that preceded the development of FTA-abs reacted to commensal antibodies, and this problem was initially solved by diluting serum (FTA-200), and later, by absorption with Reiter's treponema. It presents sensibility of 99.5% and specificity of 88.7%. In cases of autoimmune diseases it is false negative. Today, the hemagglutination reactions (MHA-Tp and HA-Tp) are used as confirming tests, mainly if the laboratory is not equipped with fluorescence microscopy.

The enzyme immunoassay (EIA) uses treponema antigen, human antiglobulin and enzyme substract, and may be modified to detect IgM.

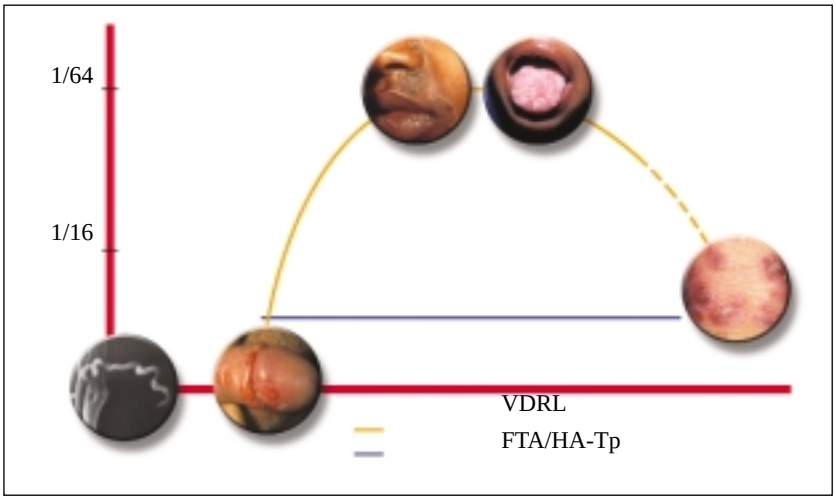
Western blot technique identifies antibodies against the following molecular mass immune determinants: 47 KDa, 17 KDa and 15 KDa. Currently, recombinant DNA techniques are used to obtain treponema antigen.[7] They are still under investigation, but will be the future commercial option, since its sensibility is 95.1% and specificity is 94.7%.

The rapid treponemic tests are extremely important to assist in making diagnosis due to possible immediate reading, thus facilitating detection. The immune chromatographic assay (Determine® Syphilis Tp) presents sensibility ranging from 93.7% to 98.4% and specificity, from 95.2% to 97.3%; it uses a colored treponema-selenium colloidal conjugate as antigen.[8,9] Other manufacturer's tests were not very effective, particularly in fingerprick whole blood investigations.[10]

Thus, it could be stated that serological diagnosis is based on two reactions (as mentioned by Assis) - lipid and treponemic - which are technically more complex. The molecular amplification reactions should be considered apart.

As early as in 1997, Zoechling et al.[11] applied the PCR technique in secondary syphilis lesions (four in six positive) and in gumma (one in seven). The technique is currently used to detect treponema antigens in primary syphilis,[12] with high sensibility (94.7%) and specificity (98.6%).[13] The RNA amplifica-

Art by Javier Comesaña



GRAPH 1: Natural history of syphilis serology

tion is even more sensitive and reveals the presence of the live microorganism. Chart 2 summarizes the most common approaches to diagnose syphilis in all stages, and graph 1 demonstrates the behavior of non-treated syphilis.

As we can verify, there has been great progress in these 80 years; however, in some situations in our daily practice, such developments seem to not exist.❑

REFERENCES

1. Assis A. O immunodiagnostico da syphilis na actualidade. Annaes Brasileiros de Dermatologia e Syphilographia, 1925; 1: 19-31.
2. Brown DL, Frank JE. Diagnosis and management of syphilis. Am Fam Physician. 2003; 68: 283-90.
3. Rotta O. Doenças sexualmente transmissíveis. In: Prado FC, Ramos J, Valle, JR. Atualização terapêutica. São Paulo: Artes Médicas; 2003. p.205.
4. Debattista J, Dwyer J, Anderson R, Rowling D, Patten J, Mortlock M. Screening for syphilis among men who have sex with men in various clinical settings. Sex Trans Infect. 2004; 80: 505-8.
5. Zielinski S, Borkhardt H. Studies on the lysozyme independence of immune immobilisation of Treponema pallidum and the frequency of lysozyme autoantibodies in syphilitic sera. J Med Microbiol. 1997; 46: 669-74.
6. Rathlev T. Haemagglutination test utilizing pathogenia Treponema pallidum for the sero-diagnosis of syphilis. Br J Vener Dis. 1967; 43: 181-5.
7. Sato NS, Suzuki T, Ueda T, Watanabe K, Hirata RD, Hirata MH. Recombinant antigen-based immuno-slot blot method for serodiagnosis of syphilis. Braz J Med Bio Res. 2004; 37: 949-55.
8. Sato NS, de Melo CS, Zerbini, LC, Silveira EP, Fagundes LJ, Ueda M. Assessment of the rapid test based on an immunochromatography technique for detecting anti-Treponema pallidum antibodies. Rev Inst Med Trop SP. 2003; 45: 319-22.
9. Diaz T, Almeida MG, Georg I, Maia SC, De Souza RV, Markowitz LE. Evaluation of Determine Rapid Syphilis TP assay using sera. Clin Diagn Lab Immunol. 2004; 11: 98-101.
10. Siedner M, Zapitz V, Ishida M, De La Roca R, Klausner JD. Performance of rapid syphilis tests in venous and fingerstick whole blood specimens. Sex Trans Dis. 2004; 31: 557-60.
11. Zoechling N, Schluepen EM, Soyer HP, Kerl H, Volkenandt M. Molecular detection of Treponema pallidum in secondary and tertiary syphilis. Br J Dermatol. 1997; 136: 683-6.
12. Wheeler HL, Agarwal S, Goh BT. Dark ground microscopy and treponemal serological tests in the diagnosis of early syphilis. Sex Trans Infect. 2004; 80: 411-4.
13. Palmer HM, Higgins SP, Herring AJ, Kingston MA. Use of PCR in the diagnosis of early shyphilis in the United Kingdom. Sex Trans Infect. 2003; 79: 479-83.

MAILING ADDRESS:
Osmar Rotta
Rua Maestro Cardim, 377 – 10º andar
01323-000 São Paulo SP