Detection of Biological False Positive Syphilis Serum Reactions

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SUMMARY

A comparative evaluation of reagin tests (Wassermann, VDRL, RPR) and fluorescent treponema antibody absorption tests (FTA-ABS) performed on blood specimens from 5 271 persons (2 493 pregnant women, 1 130 apparently healthy prospective employees, 1 345 newborn babies and 303 leprosy patients) showed that 17,2% of the pregnant women, 11,95% of the prospective employees, 19,0% of the newborn babies, and 27,2% of the leprosy patients gave positive reactions in one or more of the tests. The majority of specimens were from Cape Coloured patients. FTA-ABS tests allowed the exclusion as biological false positives of ±30% of the pregnant 'positive reactors', of ±37% of the prospective employees, 40,2% of the newborn babies, but only 1% of the leprosy patients.

The FTA-ABS test, therefore, deserves wide acceptance as the standard by which the diagnosis of syphilis is confirmed and false positive reactions are defined, although non-specificity in pregnancies, intracellular infections and auto-immune diseases occurs.

The classical reagin tests are of value in the control of treatment.

The diagnosis of syphilis depends on the correlation of all available historical and clinical data, as well as on the uniformity of a pattern of serological tests. The serodiagnosis of syphilis, started in 1906 with the introduction of the Wassermann reaction, has since steadily improved with the development of techniques and reagents.

Tests for syphilis may be divided into two main classes: non-treponemal (reagin) and treponemal (specific).

The complement fixation test for syphilis, introduced by Wassermann and his associates uses a non-specific lipoid antigen. It measures an antibody known as reagin which is not protective. This non-specific antigen is distributed widely in mammalian tissues and even in some plants. Although reagin is not a protective antibody and is measured with a non-specific antigen, it is produced by practically every patient who becomes infected with *Treponema pallidum*.

Sacks et al.[1] suggested in 1925 that an infection with *T. pallidum* damages the tissues of the host and splits off

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TABLE I. DIAGNOSTIC SEROTESTS FOR SYPHILIS

Reagin tests

| Wassermann, VDRL, RPR | Diagnostically useful, but prone to biol. false positive reactions | Excellent for control of treatment |
| Kahn, Meinicke and others | Not used in our series | |

Treponemal tests
TPI
FTA-ABS

Highly specific for diagnostic purposes, but useless for control of treatment. Only occasional biological false positive results.

a lipoidal fraction, which, acting as a hapten, combines with the protein of the treponema and stimulates the production of antibodies (reagin) which can be measured by the so-called reagin tests.

The first treponemal test using *T. pallidum* was introduced by Nelson and Mayer[2] in 1949 when they described the *Treponema pallidum* immobilisation test (TPI). In this test the serum of patients showing evidence of clinical immunity was able to render immobile living *T. pallidum* which had been obtained from primary experimental testicular lesions in rabbits.

In 1957, Deacon and co-workers[3] introduced the fluorescein treponemal antibody test (FTA) as a substitute for the expensive *Treponema pallidum* immobilisation test. The new FTA test was very sensitive but gave some false positive results because it detected antibodies in man to the essentially non-pathogenic treponemata. Hunter et al.[4] absorbed the cross-reacting antibodies from the serum by treatment with Reiter's treponema, which is a cultivable, avirulent strain of *Treponema*. This new FTA-ABS test is more sensitive and specific for *T. pallidum* and enables false positive reagin reactions to be detected. We emphasise, however, that although this test is highly specific, this specificity is not absolute.

Because of its ease and inexpensiveness, the FTA-ABS test is replacing the TPI test for confirmation of the presence of syphilis when the results of the reagin tests are in question.[3] It is also widely accepted as the standard by which false positive reactors to reagin tests for syphilis are defined.

The specificity of the FTA-ABS test is influenced by the antigenic composition of *T. pallidum,* sharing antigens with other spirochaetes, and by cross-reacting group antibodies.

Information concerning the antigenic structure of *T. pallidum* is incomplete. Limited studies have shown the

presence of proteins, polysaccharides and two different lipids. Both 19S (IgM) and 7S (IgG) antibodies are produced in man to the non-specific cardiolipin-like antigen. IgM, IgA and IgG are produced against the antigens of *T. pallidum*. IgM occurs early in infection and is of special interest for the diagnosis of congenital syphilis. Later, IgA and IgG appear; IgG persists in late infections. Therefore the FTA reaction stays positive over long periods and cannot be used as an indicator of efficient therapy.

Wilkinson and Wiseman[5] divided syphilis antibodies into two types: one specific for *T. pallidum* and other pathogenic treponemes; the other a group-reactive antibody which reacts with *T. pallidum* and with a wide variety of cultivable and commensal treponemes because of shared antigens. The group-reactive antibody is found in most normal, non-syphilitic sera at low titres. Király *et al.*[6] examined the group antibodies in human sera and in rabbit antitreponemal sera before and after absorption with *Treponema* species, using *T. reiteri, T. kazan, T. budapest, T. refringens, T. phagedenis,* and *T. minutum.* Absorptive properties varied with the individual sera, indicating the multiplicity of group antibodies and thereby the existence of at least 3 treponemal group antigens, one shared by all treponemes studied, another present only in *T. refringens,* and a third only in *T. reiteri.*

They also established that the bulk of FTA reactivity in early syphilis was due to group antibodies.

Group antibodies, therefore, may be responsible for false positive reactions, and should be removed by absorption before diagnostic tests are performed.

Steinberg[7] reported that *Treponema microdentium,* present in virtually every mouth, shares a common antigen with other treponemes, and allows for cross-reactions of antispirochaetal antibodies. Higher titres are found in individuals with moderate periodontal disease. Those with severe disease demonstrated an absence of circulatory antibody. The role of oral spirochaetes should be considered before diagnosing endemic syphilis.

Biological false positive reactions are adjudged on the basis of positive reagin and negative treponemal tests. Mostly they show an irregular pattern. All febrile and all tissue-degenerative diseases are able to produce false positive reactions. This should be kept in mind and syphilis tests should not be asked for during an acute febrile disease. Towards the end of pregnancies the rate of false positive reagin tests increases considerably. This is of special importance in countries with large uneducated population groups, where the pregnant female presents herself only at the end of her pregnancy. The burden of prophylactic treatment of thousands of biological false positive patients in countries with a high seroreactivity should not be underestimated.

FTA-ABS Test

Step 1. Absorption: Sorbent + patient's serum.

Step 2. Antigen-antibody reactions: Antigen (*Treponema pallidum*) + patient's absorbed serum (human antitreponemal globulin) = the antigen-antibody complex, which acts as antigen for step 3.

Step 3. Fluorescence of antibody-coated treponemes: Antigen-antibody complex + fluorescein conjugated antihuman globulin = fluorescence.

The essential effect of the absorption is to remove the normal threshold of group antibody. Originally ultrasonically disintegrated and then later heated, concentrated culture filtrate of Reiter treponemes was used. This is now in general use known as 'sorbent'.[5]

MATERIALS AND METHODS

Sera from 5 271 persons were tested for reagin-type antibodies. The Wassermann reaction was performed on a qualitative basis using Bacto Kolmer Cardiolipin Antigen, the VDRL-flocculation test; quantitatively, using Wellcome VDRL antigen, and the rapid plasma reagin (RPR) test using Brewer's Diagnostic Kit (RPR card tests for detection of syphilis). All sera reacting positively in one or more of these 3 tests were followed up using the FTA-ABS test for the detection of treponemal antibodies (Bacto FTA antigen code 2344). When treponemal antibodies were present, a diagnosis of syphilis was made, when they were absent, the reagin test results were regarded as biological false positive.

The 5 271 persons included 2 493 pregnant women, all inhabitants of the Western Cape (2 056 Cape Coloured, 232 Black, 201 White, and 4 Asiatic). Of these, 429 (17,2%) reacted positively with one or more of the reagin tests (18,9% of the Cape Coloureds, 15,1% of the Blacks and 2,5% of the Whites). All these figures are far above the expected 'true rate' of syphilis. The FTA-ABS test was performed on all 429 reagin reactors and was negative in 129 (30,1%) thus reducing the number of biological false positive reactors considerably. Table II shows the serological pattern of the 429 reacting pregnant women.

TABLE II. SEROLOGICAL PATTERN OF 429 WESTERN CAPE INHABITANTS (LAST TRIMESTER OF PREGNANCY)

WaR	VDRL	RPR	FTA-ABS	Absolute	Percentage
+	+	+	+	220	51,3
−	+	+	+	48	11,2
−	−	+	+	11	2,6
−	−	−	+	0	0
+	+	−	+	0	0
+	−	−	+	12	2,8
+	−	+	+	8	1,9
−	+	−	+	1	0,2
+	+	+	−	53	12,3
+	+	−	−	1	0,2
+	−	−	−	42	9,8
−	+	+	−	15	3,5
−	+	−	−	0	0
−	−	+	−	9	2,1
+	−	+	−	9	2,1
				429	100

Serological pattern / Reactor

From Table II it is obvious that regular patterns of positive reagin tests indicate a tendency towards true syphilitic reactions, while irregular reactions are mostly false positive. The Wassermann reaction was positive in 345, the VDRL in 338, and the RPR in 373 sera. This shows that the RPR reaction is oversensitive and should only be used as a screening method, but not as proof of syphilis.

In a second series, sera from 1 130 prospective employees of the Tygerberg Hospital were screened (853 Cape Coloured, 131 Black, 145 White and 1 Asiatic). Of the Cape Coloureds 14,8%, of the Blacks 4,6%, and of the Whites 2,1% were positive reagin reactors.

The total number of reagin reactors was 135 (11,95%). Of these 51 (37,8%) had negative FTA-ABS tests and were considered false positive reactors.

In comparison with the seroreactivity of pregnant women the group of male and female employees showed roughly a third less reactivity. This points to the importance of pregnancy as a cause of biological false positive syphilis reactions.

The relation between the tests performed on prospective employees is: Wassermann reaction 102, VDRL 103, RPR 113, and FTA-ABS 84 positive tests.

In a third series, sera (umbilical cord blood) of 1 345 newborn babies (1 094 Cape Coloureds, 135 Blacks and 116 Whites) were investigated. The total number of positive reagin reactors was 256 (208 Cape Coloured, 42 Black, 6 Whites). Using the FTA-ABS test, 103 could be regarded as biologically false positive. The serum of the newborns was tested for both IgG and IgM antibodies. The IgG was explained by placental transfer from the mother, the IgM was considered to be an indication of congenital syphilis. The IgG and the IgM content was defined by the Tripartigen technique (Behring-Werke). Of the 153 sera showing positive FTA-ABS IgG reactions only 6 also gave positive FTA-ABS IgM tests. Specific IgG and IgM conjugates were applied. In 2 cases, apart from the positive FTA-ABS reactions with IgG and IgM conjugates, only the Wassermann reaction was positive. This may be explained by the preference of complement fixation reactions for IgM immunoglobulins. The 6 babies showed different degrees of congenital syphilis, but 4 other babies with negative IgM FTA-ABS reactions were also, on clinical findings, suggested as cases of congenital syphilis. Similar findings were reported from Brazil.[9] Where they searched for IgM treponemal antibodies in 20 cases of congenital syphilis, using anti-IgM conjugates in FTA-ABS tests, positive results were found in 13 cases, doubtful in 4 and negative in 3. Cardiolipin tests were positive in all, as well as anti-IgG treponemal fluorescence tests. In our cases the IgM levels in sera of newborn Cape Coloured infants were mostly under 10 mg/100 ml. Positive IgM FTA-ABS reactors had IgM values between 39 and 228 mg/100 ml.

The relation between the positive tests performed on seroreactive newborn babies was: Wassermann 245; VDRL 142; RPR 156; FTA-ABS 153. The best correlation existed between the Wassermann reaction (148) and the FTA-ABS (IgG + IgM) (153). The FTA-ABS tests allowed the exclusion of 103 newborn babies (40,2%) as biologically false positive.

As leprosy is one of the diseases associated with a high incidence of positive syphilis reactions, we investigated sera from 303 lepers (178 were lepromatous, 65 tuberculoid, 59 borderline and 1 undetermined). A total of 82 (27,2%) showed positive tests in one or all reactions (Wassermann, VDRL, RPR, FTA-ABS). Of the 82 positive reactors 51 belonged to the lepromatous (28%), 21 to the tuberculoid (32%), and 10 to the borderline group (17%). Not one of these patients, all inmates of a leprosarium, showed clinical evidence of syphilis. All but 3 Cape Coloureds were from different South African Black tribes. The serological pattern was irregular and points to false positive reactions.

Contrary to the remarkable exclusion rate of biological false positive reactions in our series of pregnancies, prospective employees, and newborn babies, it was not possible to achieve much exclusion by the FTA-ABS reaction. Only 1 case could be excluded out of 55. The FTA-ABS exclusion rate, therefore, is in the neighbourhood of 2% in leprosy cases, which is not significant.

The claim that biological false positive RPR reactions have not been encountered in leprosy in limited studies (stated on p. 4 of the Brewer Diagnostic Kit pamphlet), cannot be confirmed. Our finding is that the RPR reaction is as over-reactive in leprosy as in all other conditions, making this reaction an ideal screening test.

Trials with Different Absorption Methods

Experiments were carried out using various absorption methods, in an attempt to obtain a more specific FTA-ABS reagent. We used Bacto Kolmer Cardiolipin Antigen, ultrasonically disintegrated leproma proteins, and ultrasonically disintegrated *M. tuberculosis* as sorbents in our FTA-ABS tests, run parallel with the commercially available Difco FTA-ABS Reiter spirochaete sorbent.

Cardiolipin as sorbent. A comparison was carried out on 381 routine specimens which gave positive reactions with our reagin tests. After 30 - 60 seconds absorption the sera were placed on slides prepared with FTA antigen and the normal procedure followed. One hundred and eighty-eight of the 381 sera tested with both absorption methods gave identical results, 109 were more strongly positive after cardiolipin absorption, and the remaining 84 gave weaker results after cardiolipin absorption, but were still positive after treatment with Reiter sorbent. Thirty-five reacted positively after cardiolipin absorption, but were negative after treatment with Reiter sorbent. The Reiter sorbent therefore excluded more false positive reactions.

The fluorescence microscopy picture was much clearer after cardiolipin absorption, the spirochaetes stained strongly and clearly, and there was less background interference than with sorbent absorption.

Absorption with ultrasonically disintegrated leproma proteins. Biopsy material from a typical lepromatous case was received from the Westfort Leprosarium with *M. leprae* present in abundance. After ultrasonic disintegration in a MSE disintegrator for approximately 15 minutes (amplitude: 7 μm) with cooling, the suspension was centrifuged and separated into two layers: a white fatty layer and a milky semitransparent lower layer. These were separated and tested. Microscopically all mycobacteria were disrupted. Both layers mixed easily with PBS (phos-

phate-buffered saline), which was used for dilutions of 1 : 10, 20, 50, 100 and 200. All these dilutions were used as sorbents in the FTA-ABS technique. Microscopically the pictures were good, background staining was minimal and spirochaetes well stained, but comparison with the Reiter sorbent method showed that the leproma antigen had no absorptive power. The explanation may be that in a case of lepromatous leprosy — because of a deficiency in the cellular immunology — an excess of immunoglobulins leads to a strong enhancement phenomenon. All the antigens are blocked by antibodies and therefore useless for absorption. (Our observations in this field will be published elsewhere.)

Absorption with sonicated *Mycobacterium tuberculosis.* As we could not obtain a suspension of tissue component-free *M. leprae*, we used a suspension of *M. tuberculosis*, strain H37.Rv (NCTC 7416) as sorbent. This was also not effective.

DISCUSSION

Serum tests for syphilis are positive in $\pm$ 1 - 2% of the population in areas where a high standard of living is maintained. Where yaws is endemic, nearly 50%, and where endemic syphilis is found, up to 80% of positive reactions may occur.

Surveys carried out in Africa show a high incidence of positive reactions. Serum tests are positive in $\pm20\%$ of the general population of West Africa[10] and in 5% of the educated civil servants in Dakar. Our findings are similar, with $\pm30\%$ in Ovamboland and $\pm12\%$ of the urban Cape Coloured population in the Western Cape. It appears that the percentage of positive reactors is in inverse proportion to the state of civilisation. Many of the positive reactions should be false positive, but clinical investigation of positive reactors mostly gives no clue to the identity of the disease producing the immune reaction. Elimination from treatment after detection of false positive reactions reduces the financial burden of public health services considerably.

False positive reactions today are adjudged on the basis of negative treponema tests (TPI and/or FTA-ABS), but previously on irregular patterns of reagin tests. After the introduction of cardiolipin, Brede[11] compared 45 000 blood specimens in Cologne and excluded 8% of the positive Kahn and Meinicke reactions, and 3% of the positive Wassermann reactions, as false positive on the basis of negative cardiolipin tests. This happened in 1951 in an environment free from yaws and endemic syphilis.

Since the introduction of treponemal tests, the true incidence of biological false positive reactions, especially in countries with a high incidence of positive reagin tests, is better understood. An exclusion of $\pm30\%$ of positive reactors by the FTA-ABS method is remarkable, but still not optimal, because false positive reactions can occur in tests with treponemal antigens owing to reactions with antibodies formed against others, e.g. dental spirochaetes,[7] or against nucleic acids.

The biological false positive reaction is an immunological phenomenon of considerable importance. The acute reaction of short duration is harmless to the patient, apart from the risk of misdiagnosis. The chronic reaction is associated with a wide variety of systemic diseases. A number of these patients will later develop serious diseases. The biological false positive reaction, therefore, gives an opportunity to study the development of auto-immune diseases, as well as effective forms of preventive treatment.

A further improvement in diagnostic detection may be the use of a combination of Reiter plus dental treponemes for removal of more cross-reacting antibodies.

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