MEDICAL PRACTICE

Clinical Progress

Recent Progress in Venereal Disease

Serology of Syphilis

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British Medical Journal, 1972, 2, 573-575

Infection with *Treponema pallidum* causes the production of several distinct antibodies; these are shown schematically in the Figure with the tests used for their identification.

Tests for Antilipoidal Antibody

The crude tissue extracts formerly used as antigens for the detection of this antibody have been superseded by chemically defined antigens composed of mixtures of cardiolipin, lecithin, and cholesterol. By varying the proportions of these three components the sensitivity, and, within limits, the specificity of the antigens can be adjusted. Though antilipoidal antibody (reagin) is regularly produced during syphilis and other diseases caused by pathogenic treponemes, antibodies which are virtually indistinguishable are produced in a wide variety of other conditions and may be found in normal people. It is only since the introduction of specific tests in which *T. pallidum* itself is used as the antigen that recognition of these biological false-positive (B.F.P.) reactions has been possible with any degree of certainty. The tests for reagin are now regarded as screening rather than as diagnostic procedures; positive results in the absence of definite clinical evidence of infection require confirmation by specific verification tests.

Cardiolipin tests such as the Venereal Disease Research Laboratory (V.D.R.L.) slide test have a high sensitivity in untreated early, latent, and in most cases of late syphilis; they

should give less than 1% of non-specific results with normal sera. In the rapid plasma reagin (R.P.R.) test, a modification of the V.D.R.L. test,¹ V.D.R.L. antigen is suspended in a solution containing choline chloride and finely divided carbon is added as a marker. Plasma or unheated serum is tested so that results may be obtained within a short time of blood being taken. The test appears to be more sensitive than the parent V.D.R.L. test and has a good specificity. The same antigen is used in the Automated Reagin Test (A.R.T.); the reactants are mixed mechanically and deposited on a moving strip of filter paper, positive results being shown as a clumping of the carbon particles. Specimens are tested at the rate of 100 per hour and the A.R.T. has proved to be a reliable screening test.

After treatment of primary and secondary syphilis the tests for reagin become negative in three to six months in almost all cases. The response when treatment is begun in the latent and late stages of the disease is less predictable. Many patients show a slow but progressive decline in the titre of reagin, several years elapsing before the tests become negative. In others, the tests become stabilized at a relatively fixed titre; this is not necessarily an indication for further treatment.

The nature of the antigen which stimulates the production of antilipoidal antibody in syphilis is uncertain. Whether it is a response to lipid from treponemes or to lipids set free during tissue breakdown, possibly acting in conjunction with material from *T. pallidum*, is not known. The antibody is both IgG and IgM in nature; in early syphilis IgM may predominate, as in other infections. Wright and others² have used an immunofluorescence technique to demonstrate another antibody which reacts with cell mitochondrial cardiolipin. This "cardiolipin F" antibody was found frequently in early but not in late syphilis, was usually low in titre, and disappeared rapidly after treatment. Although it can be removed from sera by absorption with cardiolipin antigen, it is thought to differ from reagin antibody.

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Biological False Positive Reactions

Transient B.F.P. reactions may be found in some individuals in many infections, especially if these are associated with fever. Quaife and Gostling³ have reported an outbreak of these reactions which was associated with serological evidence of enterovirus infection. They occur frequently after vaccination,^{4,5} but other immunization procedures do not seem so apt to provoke them.⁶ Glandular fever has often been quoted as a cause, but careful studies^{7,8} showed that the incidence was no higher than might be found in a normal population.

IgM antibodies are usually associated with the chronic type of B.F.P. reaction, which persists for longer than six months. These are often found in autoimmune disorders, being but one of the serological abnormalities found in these conditions.⁹⁻¹² They have been found in an appreciable proportion of drug addicts.¹³ A high incidence has been noted in elderly persons, and it has been suggested that ageing itself may produce them.¹⁴ Genetic factors may also play a part; Tuffanelli¹⁵ found a greater incidence of abnormalities, such as raised serum gamma-globulins, antinuclear factors, and rheumatoid factors in the relations of B.F.P. reactors than in a control series.

Tests for Group Antitreponemal Antibody

T. pallidum shares antigens in common with many other treponemes, including commensals; many normal sera contain low titres of antibody which reacts with these group antigens. This antibody is probably produced in response to the normal treponemal flora of the body but increases to much higher titres during infection with *T. pallidum* and the other pathogenic treponemes. To detect it, a protein extracted from the cultivable Reiter treponeme is used in the Reiter protein complement fixation test (R.P.C.F.T.). The group antitreponemal antibody is distinct from reagin and the combination of the R.P.C.F.T. and a sensitive test for reagin has been widely used as a screening procedure. When both types of antibody are present, the probability of a treponemal infection is very high.

Morris,¹⁶ in tests on a very large series of antenatal sera, found that the combination detected more reactors than either test alone. DeBruijn¹⁷ has reviewed evaluations of the test and holds that it has a very high sensitivity provided that a suitable complement-fixation technique is used. Förström¹⁸ found the R.P.C.F.T. less sensitive than tests for reagin in primary syphilis but that it reverted to negative more slowly than the latter tests after treatment. It was also more often positive than tests for reagin in patients with treated latent or late syphilis. Its specificity is less than the T.P.I. test but better than the tests for reagin; the incidence of false-positive reactions is less than 1% and is thought to be due to antibody reacting with a polysaccharide contaminating the protein antigen.^{19,20} This differs from the antibodies responsible for non-specific reactions in the lipoidal antigen tests and it is rare for the two antibodies to be present in the same serum. This strengthens the argument for using the two types of test in combination rather than separately.

Tests for Specific Antitreponemal Antibody

The treponemal immobilization (T.P.I.) test, described by Nelson and Mayer in 1949,²¹ was the first practical test to be developed in which living, virulent *T. pallidum* is used as antigen. It is accepted as a specific test for treponemal disease with a high sensitivity at all stages of infection except primary syphilis. Technical difficulties in its execution have limited the availability of the test and have led to a search for simpler alternative methods in which dead *T. pallidum* could serve as antigen. Techniques based on complement-fixation, agglutination, and immune adherence have been developed but have not proved satisfactory in practice.

Immunofluorescence Tests

The application of immunofluorescence methods by Deacon and his colleagues^{22,23} has led to the development of very sensitive tests for antitreponemal antibody. These are indirect tests in which serum is first applied to a fixed film of *T. pallidum*, incubated, excess serum washed off, and fluorescein-conjugated antihuman globulin added to detect union of antibody globulins with the treponemes; if this has occurred, the treponemes fluoresce under ultraviolet light.

In its first form, the F.T.A.-5 test,²² the patient's serum was diluted 1 in 5 before testing. This proved extremely sensitive, but many normal sera were reactive at this dilution because of their content of naturally occurring group antitreponemal antibody. In the F.T.A.-200 test²³ sera were diluted 1 in 200 to get above the threshold of normal antibody. This modification had a good specificity but was insensitive in comparison to the T.P.I. test. In the method used at present, the F.T.A.-A.B.S. test,²⁴ group reactive antibody is first removed from sera which are then tested at a dilution of 1 in 5. This has proved to be an extremely sensitive test which has a good specificity.

The agent used in the F.T.A.-A.B.S. test to block or neutralize the effect of group antitreponemal antibody is a heated, concentrated filtrate of medium in which Reiter treponemes have been grown. The mode of action of this "sorber" is not fully understood. The medium used to grow Reiter treponemes has itself some sorbing activity.^{25,27} The effect of sorber seems to be due in part to its high osmolarity though it does contain antigenic material from Reiter treponemes as shown by reactivity in gel diffusion tests with anti-Reiter sera.²⁸

Both group-reactive and specific antitreponemal antibodies are produced in syphilis and the former are often present at a titre which exceeds that of the specific antibody.²⁹ This is perhaps due to the infection taking place in a host which is already primed to produce the group antibody. Sorber may not neutralize the effect of all the group antibody in strongly reactive sera; this may not affect the validity of the result with such sera, but when the test is weakly reactive or is the only reactive test demonstration of the complete removal of group antibody by failure of the absorbed serum to react with Reiter treponemes while still giving definite fluorescence with *T. pallidum* is desirable. This is easier to achieve by diluting the serum in an ultrasonicate of Reiter treponemes than in the conventional sorber. There is also some evidence that absorption of sera with Reiter treponemes does not necessarily remove group reactivity against all commensal treponemes.^{30,31}

Despite these uncertainties about the sorbing agent, the F.T.A.-A.B.S. test has in practice proved a most valuable method. Evaluations³² have shown it to be more sensitive than the T.P.I. or V.D.R.L. tests at all stages of treated and untreated syphilis. This is especially true of early syphilis, in which it has been found to have a reactivity of 85%. Escobar *et al.*³³ and Garner and Backhouse³⁴ found it to give reliable results on cerebrospinal fluid. Atwood *et al.*³⁵ have shown that the F.T.A.-A.B.S. test may be reactive and the result of the T.P.I. test negative on sera from a minority of patients with proved late syphilis; this conflict may make the interpretation of results difficult. The test has a good specificity; Goldman and Lantz³⁶ found three presumed non-specific F.T.A.-A.B.S. results in tests on 250 nuns and in one of these the T.P.I. test was also weakly reactive. They emphasize that borderline reactions, of which they found five in this series, should not be given diagnostic significance. False-positive reactions have been reported with some sera containing abnormal macroglobulins³⁷ or antinuclear factor.³⁸ An atypical beaded staining of treponemes may be given by sera from some patients with systemic lupus erythematosus; this is thought to be due to antibody to deoxyribonucleic acid in these sera and to occur more commonly when the disease is active.³⁹

INHIBITION TEST

An inhibition test based on the ability of syphilitic sera to block the staining of *T. pallidum* by fluorescein-conjugated specific

serum was described by Ruczkowska.⁴⁰ This method has a good specificity but Wilkinson⁴¹ found it less sensitive than the F.T.A.-A.B.S. test.

By the use of conjugated antisera specific for the various classes of immunoglobulins it has been shown that the major antibody detected in the F.T.A.-A.B.S. test is IgG and that IgM and IgA play a subsidiary part.^{42 43} There is some evidence that antibody formed early in infection is IgM and that the test can be made even more sensitive in early syphilis if a conjugate specific for IgM is used. This procedure has also been found useful in the differentiation of congenital syphilis in the newborn baby from passive transfer of maternal antibody across the placenta. Since IgM does not normally cross the intact placental barrier, demonstration of specific IgM antitreponemal antibody in the child's circulation is presumptive evidence that it is infected—particularly if there is a rise in titre.^{44 45}

Staining by immunofluorescence methods has also been used to detect *T. pallidum* in material from early lesions. Both direct and indirect methods can be used, the reagents being absorbed with Reiter treponemes to remove group reactive antibody. The results seem as reliable as conventional examination by dark-ground microscopy.⁴⁶⁻⁴⁸

Treponemal Haemagglutination (T.P.H.A.) Test

In this test, described by Tomizawa and Kasamatsu⁴⁹ and based on work by Rathlev,⁵⁰ the antigen is a suspension of formalized tanned sheep red cells sensitized with an extract of *T. pallidum*. Cross-reacting antibodies are absorbed from sera before testing. It has not yet been fully evaluated, but preliminary reports suggest that it is both specific and sensitive. Unlike the T.P.I. and F.T.A.-A.B.S. tests it can easily be performed quantitatively and is technically much easier to set up than either of these tests.

The relative sensitivity of the various tests in untreated syphilis is compared in the Table.

Response of Specific Tests to Treatment

Unless treatment is first given during the primary or secondary stage of syphilis, both the T.P.I. and the F.T.A.-A.B.S. tests usually remain positive for many years, often for the patient's lifetime. In a minority of patients treated late in the disease the T.P.I. test may become negative while the F.T.A.-A.B.S. test

is still positive. Almost all patients treated for primary or secondary syphilis show a slow reversal of the T.P.I. test to negativity; the F.T.A.-A.B.S. test seems even slower to reverse. The behaviour of the T.P.H.A. test over a period of time is not yet certain.

The persistence of reactivity in these specific tests makes them of little or no value in the assessment of progress after treatment. Whether persistence of specific antibody is a reflection of persisting *T. pallidum* in the host is still unresolved. The value of these tests lies their use in the differentiation of positive tests for reagin due to latent treponemal disease from those due to biological false-positive reactions and in the diagnosis of late manifestations of syphilis when the tests for reagin are negative or inconclusive. A positive result in the T.P.I. or F.T.A.-A.B.S. tests is an indication of treponemal infection past or present; it does not necessarily mean that the disease process is active. Indeed, the tests for reagin may offer a better guide to activity; this may be suspected if the V.D.R.L. test is reactive at a dilution of 16 or above, particularly if there is a rising titre. No test at present available will distinguish syphilis from yaws, pinta, or endemic non-venereal syphilis.

Relative Sensitivity of Tests in Untreated Syphilis

Test	Stage of Syphilis			
	Primary	Secondary	Latent	Late
V.D.R.L., A.R.T.	..	+++	++	++
R.P.C.F.T.	..	+++	++	++
T.P.I.	..	+++	+++	+++
F.T.A.-A.B.S.	..	+++	+++	+++
T.P.H.A.*	..	+++	+++	+++

*Based on early reports.

Chlamydia and Non-Specific Urethritis

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British Medical Journal, 1972, 2, 575-577

Ever since Neisser⁹⁰ described the gonococcus in discharge from the eye and from the genital tract the history of urethritis has been linked with that of conjunctivitis.^{91 92} Organisms that infect the genital mucous membranes also attack the conjunctiva, where their effects may be studied more readily than in the

genital tract. Until Chlamydia was cultured, 50 years after the agent was first described in smears from the eye⁹³ and then from the genital tract, its significance remained controversial despite many studies.⁹⁴ T'ang *et al.*⁹⁵ cultured the causative agent of trachoma in the yolk sacs of fertile hen's eggs. The conjunctiva of volunteers was inoculated with the agent and trachoma resulted.^{96 97} Chlamydia was isolated from the eye of a baby suffering from ophthalmia neonatorum and from the cervix of the mother of such a baby by Jones *et al.*⁹⁸ It was also recovered from the cervix of a mother of twins suffering from ophthalmia neonatorum; this mother was suffering from trachoma and one of the twins developed that condition.⁹⁹ Jones and Collier^{100 101} produced trachoma by inoculating the conjunctiva of an adult with agent from the eye of a baby suffering from ophthalmia neonatorum; this agent was of genital

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