VDRL TEST AND ITS INTERPRETATION

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Summary

VDRL slide test is the most commonly used serological test for syphilis. It is an important adjunct to diagnosis and management of syphilis. Its merits and demerits, indications, and response to treatment are discussed. Various, recently reported causes for false reactions to this test are reviewed. Use of this test during pregnancy to eliminate congenital syphilis is stressed. How this test will be useful in the diagnosis and management of neurosyphilis and congenital syphilis is briefly discussed.

KEY WORDS: VDRL test, Biological false positive reactions, Serological test for Syphilis.

Unlike the darkfield examination which provides the absolute diagnostic test for syphilis[1], except, probably when done on mouth lesions[2], positive serological tests for syphilis (STS) must be accurately evaluated and interpreted. A proper and intelligent interpretation is essential on the part of the physician who is to treat syphilis and not the VDRL test. A reactive VDRL (Venereal Disease Research Laboratory) test in a patient with no clinical, historical or epidemiologic evidence of syphilis may cause much concern to both the physician and the patient. The difficulty to interpret the result of the test often results in diagnostic confusion. The reactivity of serum may be unrelated to syphilis or stigma of a past infection.

The serological tests for syphilis (STS) may be broadly divided into specific and nonspecific tests. During the course of infection with *Treponema pallidum* and in some other acute and chronic conditions, 'reagin' - an antibody like substance[3] appear in the sera of the patients. This non-treponemal reaginic antibodies produced in syphilis are of both Ig G and Ig M[4] origins. This reagin can be detected either by flocculation test or complement fixation test. The latter is time consuming and expensive and so only rarely done nowadays. Among the flocculation tests, though there are various newer techniques like rapid plasma reagin (circle) card test, automated reagin test etc, the VDRL slide test which is cheap and easy to perform has won general acceptance as a test of high sensitivity[5]. In the flocculation test, cardiolipin, lecithin and cholesterol as antigens combine with reagin to form aggregates that can be observed as floccules. The highest dilution of the serum of a patient in which visible flocculation can be detected is the basis of the quantitative VDRL test. These tests for reagin are best called non treponemal tests, but often have been referred to by the

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Received for publication on 6—6—1983.

less specific term 'serological test for syphilis'[6]. In the specific tests for syphilis the *Treponema pallida* (live, dead or disintegrated) are used as antigen. The treponema pallidum immobilisation test (TPI), fluorescent treponemal antibody absorption test (FTA-ABS), treponema pallidum haemagglutination test (TPHA), Fluorescent treponema – absorption Ig M test (FTA Ig M) etc are the specific tests. Though labelled as specific, these tests also may sometimes give false positive reactions[7,8]. The accuracy of the various serological tests is defined by sensitivity and specificity. Sensitivity is the percentage of positive results obtained with the test on syphilitic sera while specificity is defined as the percentage of negative results obtained in a non syphilitic population[9].

Serology in different stages of syphilis

The reagin starts appearing in the serum, 5 to 8 weeks only after acquiring the infection with *Treponema pallidum*. The VDRL test becomes reactive in only 2/3 of cases of primary syphilis[3,10]. In the series reported by Deacon et al[11] the VDRL test results were non-reactive in 24% of the untreated cases of primary syphilis. The test remains non reactive throughout the incubation period of syphilis[12]. Within the first week or more after the development of the chancre the serology is likely to remain non reactive. This stage is called the seronegative phase of primary syphilis. At this stage the diagnosis of syphilis depends on the demonstration of *Treponema pallidum* by dark field microscopy. Even though the FTA-ABS test may be negative in a few during primary syphilis[2], it is more sensitive than the VDRL test at this stage of the disease[13]. The VDRL test in primary syphilis thus may be nonreactive or reactive in low titer mostly but at times in high titre (more than 1 : 32)[6;14]. If the dark field examination is negative and clinical suspicion with regard to syphilis is low in a particular lesion, a reactive serology of 1 : 4 or 1 : 8 dilution should not tempt the physician to treat the patient forthwith for syphilis. The darkfield examination should be repeated daily and any treponemicidal drug withheld for the time being. If the sore is due to syphilis, the VDRL titer may rise to fourfold or more within three weeks. If on the other hand the serology was of a false nature, the titer of reactivity is likely to decline or test may become non-reactive. The specific tests even though confirm the syphilitic aetiology of the reaginemia, it does not indicate the activity of the disease. It simply reveals that the patient has had syphilis. While the FTA-ABS and RPCF tests become positive earlier than the VDRL test, the TPI test becomes positive only a few days after the VDRL test becomes reactive[3]. After treatment, in primary syphilis, the VDRL titer usually falls to non-reactivity within 3 to 6 months. The speed with which seroconversion occurs depends on the duration of the primary chancre prior to the treatment and also on the height of pretreatment titer[14]. Higher the initial titer, longer the time required for reaching seronegative phase of chancre, the test is likely to remain non reactive throughout. Sometimes seropositivity may be detected after specific treatment in a seronegative primary chancre[3].

In the secondary stage of syphilis the VDRL test is almost always reactive and usually the titer is very high. But recently, a high number of false negative STS has been reported from Uganda even in secondary syphilis[15]. Because of the high concentration of reagin in the serum during this stage, and occasionally in late active syphilis[16], a false negative result to VDRL test may occur in 1 to 2% of cases[17].

This is due to prozone (prezone) phenomenon in serology. This can easily be overcome by proper dilution of the serum. If therefore the clinician strongly suspects secondary syphilis in a case where the VDRL test is reported as nonreactive, the possibility of prozone phenomenon should be kept in mind and necessary advice should be given to the serologist to repeat the test with a diluted sample of serum. After adequate treatment of secondary syphilis, the titer falls either by a 'lytic' response where there is a rapid fall in titer to nonreactivity within a few months or by a 'slow' response where the fall in titer is gradual throughout, never reaching negativity but remaining reactive in a low titer for a long time, sometimes for years. In most cases if treatment is given during secondary stage, seronegativity should be achieved within two years[18].

Latent syphilis is that stage of syphilis where the STS is reactive in the absence of clinical evidence of any stage of syphilis[1]. Before labelling as 'latent', a specific test and a CSF study should be shown positive for syphilis. The latent syphilis may be 'early' if the infection is of less than two years duration and 'late latent' if it is of more than two years. In practice however it is often difficult to ascertain whether latent stage is early or late. Patients with frequent "exposures" may not be able to reveal the exact duration of their infection. But if the fall in titer of reagin, after specific treatment, of latent syphilis is rapid, it is likely to be early latent infection. In the late-latent stage the titer may not fall rapidly and in some cases may not fall at all.

Follow up of serology in early syphilis :- Any patient with a genital lesion or with a history of exposure to STD, should be subjected to blood VDRL test even if one does not suspect syphilis. This is important because it establishes a baseline of reactivity from which future specimens may be evaluated. Thus, a rising titer may be indication of a recently acquired infection, a reinfection or a relapse in a 'serofast' individual[2]. In early stages of syphilis, after adequate treatment the patient should be followed up with repeated STS. Usually it is repeated once in a month for three months, then once in three months for six months and then every six months till the end of two years of acquiring infection. When tetracycline is used for treatment, this period of follow up must be prolonged, since the efficacy of this drug in syphilis is still not fully evaluated. A transient rapid rise in titer may occur immediately after treatment with a treponemicidal drug due to sudden destruction of the treponemes. As far as infection with *Treponema pallidum* is concerned, resistance to penicillin, is rare in India. If treatment is inadequate, a relapse may occur. In the absence of any clinical lesions, the reagin titer alone may rise steadily in a patient, on follow up. This is called 'serorelapse'. A one tube dilutional change in titer (for e.g. 1 : 8 to 1 : 16) falls within the range of laboratory variation and is not significant[19]. But if there is a four fold increase in titer (e.g. 1 : 8 to 1 : 32) treatment failure or relapse should be suspected presence of active disease. In such cases treatment should be reinstituted[6]. In some cases after specific treatment, the titer falls to the lowest level of 1 : 4 or 1 : 8, dilution however reaching negativity, but showing fluctuations between 1 : 2 and 1 : 8. The exact mechanism of this is not clear. This does not indicate need for further treatment and any further antisyphilitic treatment given will not make the VDRL non reactive in these cases. A patient on follow up after adequate treatment may sometimes be wrongly labelled as having

latent syphilis. The patient who is on 'follow up, serology, after treatmant, may consult a new physician for his reactive VDRL test. A history of treatment for syphilis in the past should be excluded before diagnosing latent syphilis.

The sensitivity of VDRL test decreases considerably in late latent and late syphilis and non reactivity in as high as 30% of cases has been noted[11]. In patients with active gummas, the VDRL test is usually reactive in high titers[12]. In cardiovascular and neurosyphilis the VDRL test is reactive in only two thirds of the cases, non reactivity occurring less often in cardio vascular syphilis than in neurosyphilis[20,21]. Even when reactive, the titer of reagin is usually low and will not fall rapidly after specific treatment, even after years. No amount of additional treatment will revert the serology to non reactive state and attempts should not be made to obtain sero negativity in such cases[19].

Congenital syphilis :- A reactive serology in a child born to a syphilitic mother need not always be due to syphilitic infection of the neonate. It can be due to passive reaginaemia where the reagin is simply transferred through the placenta to the new born. In such cases, the titer usually declines sharply in the first two to three months of life. If there is true neonatal syphilis the titer rises progressively. In true syphilitic infection of the new born the titer of reagin will be more in the cord blood than in the maternal blood[2]. A gradual fall in titer in a child need not always mean passive reaginemia. It occurs also when there was a syphilitic infection in utero but because of treatment administered to the present mother through whose placenta the child also received adequate therapy. A non reactive VDRL test in a new born does not always exclude possibility of congenital syphilis. A pregnant woman getting infected late in pregnancy may give birth to a newborn who is initially non reactive serologically but becomes reactive some months after delivery[22,23]. In such cases long follow up is essential.

Active infection of the new-born can be detected by performing the FTA-ABS-Ig M test[24]. Under normal conditions Ig M is not transferred across the placenta. The foetus or neonate can produce Ig M antibody in response to syphilitic infection. The test is based on the assumption that since Ig M antibodies do not traverse the intact placenta, any Ig M antibody detected by FTA-ABS-IgM test for neonatal sera has to be attributed to infection in the new born[2]. Serial tests are necessary because some babies may show a delayed onset, the IgM-FTA test being negative at birth but becoming positive after an interval of several weeks[25]. In late congenital syphilis the titer is usually low, but not necessarily[26]. After treatment, the titer of reagin is not related to the activity of the disease and fluctuation between weak and strong reactivity occurs. The reason for this wide fluctuation is not known. There is no indication to treat such cases.

Cerebro spinal fluid and the VDRL test :- A reactive VDRL test in CSF is almost always an indication of neurosyphilis[2,16], but a non reactive test does not exclude neuro syphilis[27]. After treatment of neurosyphilis, though the abnormal cell count tends to disappear within a few months the reagin in CSF persist for years. So VDRL test is of little value in assessing the prognosis of neurosyphilis after treatment. Recently, various cytological, immunological and chemical tests have been suggested for establishing the activity of neurosyphilis[28].

False reactive VDRL test in CSF is very rare. A patient suffering from syphilis, who also has meningitis due to some other cause, may have a positive CSF finding due to passage of reagin from blood through the choroid plexuses. False reactions in CSF has been reported in sub arachnoid haemorrhage, sarcoidosis, collagen vascular diseases[29] and spinal cord tumours[30]. After a bloody lumbar puncture, false reaction may occur in CSF, due to contamination by blood which may be serologically reactive.

Blood transfusion and VDRL test: It should be routine to perform VDRL test before collecting blood from a donor for transfusion to a recipient. A reactive VDRL in a sample of blood from a donor causes much confusion. It should be kept in mind that a reactive VDRL test reveals only the presence of reagin and not necessarily the presence of *Treponema pallida*. If the blood is kept well refrigerated at 4°C for 72 hours continuously, spirochaetes, even if present, become inactivated.

Pregnancy and the VDRL test: All women, in the first trimster of their pregnancy should be subjected to blood VDRL tests. This simple step will help to completely eliminate congenital syphilis[12]. It is not uncommon to get biological false positive reactions in these cases. Tuffanelli et al[31] reported that almost a third of their 211 acute false positive reactors were pregnant. Even the specific tests may give false results during pregnancy[32]. Considering the risk to the foetus, it is always justified to treat a pregnant woman who is reactive serologically, even if a false positive reaction is suspected.

False positive reactions to VDRL test: False positive serological test results have been reported to account for as few as 3% and as many as 40% of reactive tests[6]. There is no serologic test or specific test to differentiate whether the positive serology in a patient is due to venereal or non venereal syphilis. A detailed clinical, historical and epidemiological study may help in the differentiation. Recently, it has been viewed that since non venereal syphilis is acquired during childhood, the presence of FTA-Ig M test in a patient suggests venereal rather than nonvenereal syphilis. Technical errors can occur in recording the results of STS in laboratories. A series of tests are indicated when physician does not suspect syphilis in a patient whose blood VDRL is reported reactive. A specific test also may be performed. The biological false positive reactions (BFP) may be of acute or chronic type. Acute transient reaginemia usually in low titer (1 : 1 to 1 : 4)[33] occurs in viral fever, filariasis, tuberculosis, malaria, L. G. V. herpes simplex, pregnancy, chlamydial infections, typhus fever, relapsing fever, ratbite fever, measles, chicken pox, sub acute bacterial endocarditis and infective hepatitis. Acute BFP reactions may occur after immunisation against bacterial and viral infections[34] and also after use of certain drugs for hypertension[2]. In acute BFP reactions the test becomes nonreactive within a short period usually six months. In chronic BFP reactions, the titer is usually high and persist for more than 6 months and sometimes for years. This creates a greater diagnostic problem and sometimes this false reaction in a patient may be a manifestation of an underlying serious disease. It is the responsibility of the physician to determine the possible cause of this type of reaction. These patients should be subjected to detailed investigations including tests to detect antinuclear antibodies, rheumatoid factor, hyper gammaglobulinemia, LE cells and other tests as indicated. False positive

reagin test occurs in a high percentage of narcotic addicts[35] and it persists for over a year after the patient stops taking the drug[36]. Kraus et al in their study found that 24 out of 150 patients with SLE had a positive VDRL test[8]. Relatives of these patients also may show false positive results to STS[37]. Chronic BFP reactions occur also in 8 to 28% of patients with leprosy[38], majority of them being lepromatous cases[39]. The presence of false positive reagin test increases with advancing age and 10% of people over 70 years of age will have false positive reaction to VDRL test[40].

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