Influence of genital herpes on results of fluorescent treponemal antibody absorption test

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SUMMARY Both the fluorescent treponemal antibody absorption (FTA-ABS) test and Venereal Disease Laboratory (VDRL) test for syphilis were performed routinely on 113 men with histories of genital ulcerations. The difference in negative VDRL and borderline FTA-ABS results between patients with and without herpes simplex virus in their genital ulcers and no evidence of previous or untreated syphilis was not statistically significant. Furthermore, use of the FTA-ABS test as a confirmatory rather than as a screening test eliminates false-positive, borderline, or reactive results in patients with non-syphilitic ulcers.

Introduction

The fluorescent treponemal antibody absorption (FTA-ABS) test is highly specific and false-positive reactions are rare (Mackey *et al.*, 1969, Goldman and Lantz, 1971). False-positive reactions occur mainly in conditions associated with increased or abnormal immunoglobulins such as autoimmune or connective tissue disease (Catterall, 1972; Tuffanelli, 1973; McKenna, *et al.*, 1973). Borderline or false-positive results have also been reported in cases of drug addiction (Kaufman *et al.*, 1974), smallpox vaccination (Jacobs *et al.*, 1971), pregnancy (Drew and Sarandria, 1975), and drug-induced lupus erythematosus (Monson, 1973).

Wright *et al.* (1975) reported false-positive FTA-ABS results in 32 patients with genital herpes simplex virus (HSV) infection and no historical or clinical evidence of previous or untreated syphilis. All of these patients had non-reactive results to rapid plasma reagin (RPR) and *Treponema pallidum* haemagglutination (TPHA) tests. However, six (18.8%) patients had reactive results to the FTA-ABS tests. Wright *et al.* (1975) speculated that since HSV is a DNA virus the infected host may form anti-DNA antibody. If the commercially prepared sorbent fails completely to adsorb this antibody it may attach to the DNA of the treponemes used in the FTA-ABS reaction and thus be detected by immunofluorescence.

Clinicians lacking access to darkfield microscopy and culture for HSV must base a diagnosis of syphilis on the clinical picture and on the results of serological tests. In such instances Duncan *et al.* (1974) have emphasised the use of the FTA-ABS test. However, false-positive FTA-ABS reactions in patients with genital herpes infection could lead to an incorrect diagnosis of syphilis.

This paper reports the results of the FTA-ABS test, with commercial reagents, in a group of patients with genital ulcers. Data from patients with and without genital herpes are compared and these results discussed in relationship to those published by Wright *et al.* (1975).

Materials and methods

STUDY POPULATION

The study was conducted at the Detroit Social Hygiene Clinic from September 1973 to March 1975. One hundred and thirteen consecutive men with penile ulcerations underwent a standardised interview and examination after written, informed consent. Of these, 13 were Caucasian and the remainder were black.

MICROBIOLOGICAL STUDIES

Secretions were collected with sterile rayon-tipped swabs and cultured for aerobic and anaerobic bacteria, mycoplasma, yeasts, and fungi, as reported by Chapel *et al.* (1978). Material for virological studies was scraped from the ulcer base with a sterile surgical blade, placed in virus transport

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medium, and immediately sent to the laboratory. This material was used to inoculate baby hamster kidney cells (BHK-21/13), and HSV was identified by the neutralisation technique of Tokumaru *et al.* (1969). In addition, ulcer secretions were examined for treponemes by darkfield microscopy.

IMMUNOLOGICAL STUDIES

Immunological studies were performed on serum obtained from patients at the initial visit and again at 10 to 14 days. Fresh sera were tested by the Venereal Disease Research Laboratory slide test (US Center for Disease Control, 1969), and the FTA-ABS test was performed by the technique of Hunter *et al.* (1964), and with materials, sorbent, and antigen supplied by Baltimore Biological Laboratories.

Circulating HSV antibodies were measured by the indirect haemagglutination test. The McIntyre VR₃ strain was used to prepare type 1 antigen and the MS strain for type 2 antigen, according to the methods of Stewart and Hermann (1976) and Palmer *et al.* (1974).

Results

A detailed report of the microbiological flora recovered from the ulcers of 100 of the 113 men has been reported by Chapel *et al.* (1978). HSV was cultured from the ulcers of 41 men, and three more men with herpetic ulcers had negative culture results, but their paired sera showed a four-fold or greater rise in HSV antibody titres. This combination of clinical and serological findings supported a diagnosis of genital herpes. Of these 44 patients with genital herpes, six had concomitant gonorrhoea, six non-specific urethritis, two infection with *Haemophilus ducreyi*, and two infection with *Treponema pallidum*.

Sixty-nine men had no laboratory evidence of genital herpes, but 12 of them had primary syphilis, and three more had secondary syphilis with persisting chancres. In addition, seven men had lesions of probable traumatic origin, two had ulcerated, fixed drug reactions, and four had, respectively, primary cutaneous gonococcal infection, Group A streptococcal pyoderma infection, staphylococcal pyoderma infection, and an ulcerated *Candida albicans* balanitis. The cause of the ulcers in the remaining 41 men was not established. Sexually transmitted diseases unassociated with the genital ulcers were diagnosed in 20 of the 69 men. Of these 20 patients, 10 had concomitant gonorrhoea, seven non-specific urethritis, and three condyloma acuminatum.

Sera from all 113 patients were examined by the VDRL slide and FTA-ABS tests. Results to both the VDRL and the FTA-ABS tests were negative in 36 men with genital herpes and reactive in five patients with genital herpes (Table 1). An additional two men with HSV had borderline

Table 1 *Results and case histories (on first visit) of eight patients with genital herpes and positive results to serological tests for syphilis*

Case no.	Results of serological tests		History of syphilis
	VDRL	FTA-ABS	
5	WR	NR	None
78	NR	BL	None
43	R (1:4)*	R (+2)†	Primary
79	NR	R (+1)	Primary
29	WR	BL	Previously treated primary
85	R (1:4)	R (+4)	Previously treated primary
4A	NR	R (+2)	Previously treated primary
3A	R (1:16)	R (+4)	Previously treated early latent

WR = Weakly reactive

NR = Non-reactive

BL = Borderline reactive

R = Reactive with titre (VDRL)* or degree of reaction (FTA-ABS)†

reactive results in the FTA-ABS test. The five patients with reactive results and one of the two with borderline results to the FTA-ABS test had histories of current or treated syphilis. Only one patient (Case 78) had borderline results to the FTA-ABS test, negative results to the VDRL test, and no evidence of untreated or treated syphilis. On repeat testing 21 days later the results of both the VDRL and FTA-ABS tests were non-reactive.

One or both serological tests for syphilis gave reactive results in 30 of the remaining 69 men without cultural or serological evidence of HSV (Table 2). Of these 30 men 24 had histories of treated or untreated syphilis. Two other men had reactive results to the VDRL test but non-reactive results to the FTA-ABS test, and four others had non-reactive results to the former and borderline results to the latter. Three of the latter four men had negative results to the serological tests when repeated, while the other defaulted. The difference in negative results and borderline results to the VDRL and FTA-ABS tests respectively between men with and without HSV in their genital ulcers and no evidence of previous or untreated syphilis was not significant when analysed by the Fisher's Exact test using the one tail test ($P=0.2371$).

Discussion

Both the VDRL and FTA-ABS tests were performed routinely on each of the 113 men who participated

Table 2 Results and pertinent histories (at first visit) of 30 patients with genital ulcers and negative results to tests for herpes simplex virus but positive results to serological tests for syphilis

Case no.	Results of serological tests		History of syphilis
	VDRL	FTA-ABS	
24	NR	BL	None
31	NR	BL	None
42	WR	NR	None
48	WR	NR	None
59	NR	BL	None
64	NR	BL	None
32	R (1:16)*	R (+4)†	Primary
38	R (1:16)	R (+4)	Primary
39	R (1:4)	R (+3)	Primary
51	R (1:32)	R (+2)	Primary
52	R (1:2)	R (+1)	Primary
57	R (1:16)	R (+4)	Primary
60	R (1:32)	R (+2)	Primary
89	R (1:4)	R (+2)	Primary
91	WR	R (+4)	Primary
96	R (1:8)	R (+4)	Primary
99	R (1:2)	R (+3)	Primary
13	R (1:128)	R (+4)	Secondary
22	R (1:128)	R (+4)	Secondary
66	R (1:64)	R (+4)	Secondary
4	WR	R (+1)	Previously treated
46	NR	R (+1)	Previously treated
65	R (1:1)	R (+2)	Previously treated
69	NR	R (+1)	Previously treated
83	NR	R (+3)	Previously treated
90	NR	R (+4)	Previously treated
101	NR	R (+4)	Previously treated
9A	R (1:2)	R (+4)	Previously treated
1	R (1:2)	R	Previously treated
40	WR	R	Previously treated

WR = Weakly reactive

NR = Non-reactive

BL = Borderline reactive

R = Reactive with titre (VDRL)* or degree of reaction (FTA-ABS)†

in the study with a history of genital ulcerations. Five of the patients who had neither treated nor untreated syphilis gave borderline reactive results to the FTA-ABS test, but non-reactive results to the VDRL test, both of which tests gave negative results in the four patients who returned for follow up. These results are similar to those of Jaffe (1975), who reported that most patients with negative and borderline reactive results to the VDRL and FTA-ABS tests respectively have non-reactive results to serological tests on repeat examination. Only a small percentage of these patients had untreated syphilis.

In our patients, the difference between negative and borderline results to the VDRL and FTA-ABS tests, respectively, in men with and without HSV in their genital ulcers, and no evidence of previous or untreated syphilis, was not statistically significant. The difference between our results and those reported by Wright *et al.* (1975) may be due, as they suggested, to sorbent that failed to remove viral

anti-DNA antibody which then attached to the DNA of the treponemes. Indeed, Wilkinson and Ferguson (1968), and Wilkinson and Wiseman (1971), and Mackey *et al.* (1969) found lower false-positive results to the FTA-ABS test with a whole Reiter treponeme 'sonicate' than with the culture fluid extract 'sorbent'. The commercial sorbent (Baltimore Biological Laboratories) used in this study provided greater specificity than the commercial sorbent used by Wright *et al.* (1975). Previously mentioned concomitant diseases or problems with laboratory techniques as reported by Sparling (1971), Elsas (1972), Pien *et al.* (1976), and McKenna *et al.* (1973) could perhaps explain some of the false-positive results.

Burns (1975) reported a series of 56 clinically healthy persons with no history of syphilis and negative results to the VDRL test but borderline or reactive results to the FTA-ABS test. Twenty (35.7%) of these patients spontaneously developed non-reactive results to the FTA-ABS test over a period of eight months or less. Further, Jaffe (1975) found that when the FTA-ABS test is used to screen for syphilis it gives an increased incidence of false-positive reactions. Proper use of the FTA-ABS test as a confirmatory rather than as a screening test eliminates false-positive borderline or reactive results to the FTA-ABS test in patients with non-syphilitic genital ulcers. Nonetheless, additional reports are necessary to evaluate further any possible link between genital herpes infection and apparent false-positive reactive results to the FTA-ABS test.

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