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False-Positive Reactions in the Rapid Plasma Reagin-Card, Fluorescent Treponemal Antibody-Absorbed, and Hemagglutination Treponemal Syphilis Serology Tests

CHRISTOPHER R. PETER,[1]* MURIEL A. THOMPSON,[1] AND DAVID L. WILSON[2]

San Diego County Public Health Laboratory, San Diego, California 92101,[1] and Difco Laboratories, Detroit, Michigan 48232[2]

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Sera from 628 nonsyphilitic individuals were tested with the Rapid Plasma Reagin-Card, Fluorescent Treponemal Antibody-Absorbed, and Hemagglutination Treponemal Test for Syphilis tests to ascertain the comparative specificity of these tests. Many sera were also tested with the quantitative Venereal Disease Research Laboratory test. Sera included in the study were from both normal individuals and patients with a variety of illnesses and conditions. The Hemagglutination Treponemal Test for Syphilis gave the lowest overall percentage of false-positive reactions (1.6%), followed by the Fluorescent Treponemal Antibody-Absorbed test (3.3%) and the Rapid Plasma Reagin-Card test (10.8%).

Frequently during the course of screening patients undergoing physical exams or at the time of hospital admission a physician will order a serological test for syphilis which is unexpectedly positive. Understandably, physicians are reluctant to treat a patient for syphilis in the absence of appropriate history or symptoms and especially when there is another underlying illness or condition which may be suspected of causing a false-positive serological test for syphilis (6).

We undertook a comparative study of sera from 628 nonsyphilitic patients to determine the false-positive rates, using a variety of serological tests for syphilis. Tests used were the (i) Rapid Plasma Reagin test (RPR), (ii) Venereal Disease Research Laboratory test (VDRL), (iii) Fluorescent Treponemal Antibody-Absorbed test (FTA-ABS), and (iv) Hemagglutination Treponemal Test for Syphilis (HATTS).

MATERIALS AND METHODS

Sera were obtained from 299 San Diego County and City Civil Service employees at the time of pre-employment physicals and from 149 prenatal patients attending clinics at the San Diego County Department of Public Health. Sera from 100 patients with clinical and serological evidence of nonsyphilitic infectious diseases were obtained from the serology section of this laboratory; these sera had originally been submitted to this laboratory by local physicians and clinical laboratories. Eighty sera from patients diagnosed as having one or more biological false-positive (BFP) syphilis serology tests were obtained from health department venereal disease clinics and from laboratories throughout San Diego County for testing. The BFP determinations were based on discussions of the test results with the attending physicians, and reliance was placed on their evaluations of the histories and conditions of each patient.

The RPR, FTA-ABS, and HATTS tests were performed on all specimens included in the study. The VDRL test was performed only on sera from patients in the BFP group. The RPR, quantitative VDRL, and FTA-ABS were performed according to standard procedures with Center for Disease Control-specified controls. The HATTS test was performed using kits donated by Difco Laboratories. The procedures used for this test have been described in detail elsewhere (B. B. Wentworth et al., Sex. Transm. Dis., in press). Briefly, 0.1 ml of *Treponema pallidum*-sensitized turkey erythrocytes was added to microtiter U plates containing 0.025 ml of a 1:16 dilution of heat-inactivated serum in HATTS test diluent (0.85% NaCl solution with 1.6% fluorescent treponemal antibody sorbent, 10.8% normal human serum, and 0.24% sodium azide). In addition, 0.1 ml of a nonsensitized erythrocyte suspension was added to 0.025 ml of serum diluted as above. In each test the final dilution of serum was 1:80. Reactive (diluted 1:80 through 1:5,120 in HATTS test diluent) and negative (diluted 1:80) control sera were also included each day of testing. After gentle agitation, plates were incubated for 1 h at room temperature (25 ± 5°C) and read for agglutination.

RESULTS

The FTA-ABS test was shown to give the highest rate of false positives in all of the patient groups except the BFP and prenatal groups (see Table 1). The two tests using cardiolipin antigens, the RPR and VDRL, gave by far the highest false-positive rates (80.0 and 91.3%, respectively) in the BFP group. In each of the four

groups, the HATTS as compared to FTA-ABS gave the lower false-positive rate. Overall, the HATTS test showed only 1.6% false positives as compared to 3.3% false positives and 4.0% borderlines with the FTA-ABS.

Among the group of patients with infectious diseases other than syphilis, 6% of the sera showed false-positive and 12% of the sera showed borderline reactive FTA-ABS results (Table 2). In contrast, only a 1% false-positive rate for the RPR and a 2% rate for HATTS were observed. Sera from only 10 of 628 patients (1.6%) included in the study gave positive HATTS tests, and 8 of these had either positive or borderline FTA-ABS results.

Approximately one-half of the patients in the BFP category (see Table 3) had no noted illness or symptoms. Two of their sera gave false-positive reactions with all tests done. Two patients with autoimmune diseases (specifically, rheumatoid arthritis) also had all false-positive tests.

DISCUSSION

As another recent study has shown (2), our findings indicate that treponemal tests, especially the FTA-ABS, are too sensitive to use in screening for syphilis. Although the RPR was positive in 11% of sick and healthy nonsyphilitic patients included in this study, the majority of these had negative treponemal tests.

A most interesting observation was that sera from three of seven genital herpes patients had borderline FTA-ABS results (Table 2). Previous studies by Brown and Stenchever (2) and Wright et al. (10) have reported that sera from approximately 40% of genital herpes patients gave false-positive or borderline FTA-ABS results, al-

TABLE 1. *False-positive results in serological tests for syphilis: RPR, FTA-ABS, and HATTS testing of 628 nonsyphilitic individuals*

Category	No. of individuals	Avg age (yr)	% False positive				
			RPR	VDRL	FTA-ABS		HATTS
					Positive	Borderline	
Civil Service employees	299	38.4	0.3	ND[a]	1.6	3.6	1.3
Prenatal patients	149	23.8	1.3	ND	0	0.6	0
Patients with viral, mycoplasmal, chlamydial, and fungal infections	100	28.4	1.0	ND	6.0	11.0	2.0
BFP	80	32.8	80.0	91.3	12.5	2.5	5.0
All groups	628	32.6	10.8		3.3	4.0	1.6

[a] ND, Not done.

TABLE 2. *False-positive reactions in the RPR, FTA-ABS, and HATTS tests in 100 patients with viral, mycoplasmal, chlamydial, or fungal infections*

Disease agent	No. of cases	No. of cases showing:			
		RPR positive	FTA-ABS		HATTS positive
			Positive	Borderline	
Adenovirus	6	0	0	1	0
Coxsackie virus B	20	0	4	1	1
Cytomegalovirus	8	0	0	2	1
Herpes simplex virus (genital infections)	7	1	0	3	0
Influenza virus	10	0	1	1	0
Mumps virus	1	0	0	1	0
Parainfluenza virus	2	0	1	0	0
Respiratory syncytial virus	1	0	0	0	0
Rubella virus	1	0	0	1	0
Rubeola virus	17	0	0	1	0
Varicella zoster virus	1	0	0	0	0
Mycoplasma pneumoniae	20	0	0	1	0
Chlamydia psittaci	1	0	0	0	0
Blastomyces dermatitidis	1	0	0	0	0
Coccidioides immitis	4	0	0	0	0

TABLE 3. *False-positive reactions in the RPR, VDRL, FTA-ABS, and HATTS tests in 80 patients and clinically normal individuals with BFP reactions*

Disease or condition	No. of individuals tested	No. of cases showing:				
		RPR positive	VDRL positive	FTA-ABS		HATTS positive
				Positive	Borderline	
Other venereal disease (gonorrhea, herpes)	3	3	3	0	0	0
Autoimmune diseases (thyroiditis, positive ANA test, rheumatoid arthritis)	5	5	5	3	0	2
Cancer (colon carcinoma)	1	1	1	0	0	0
Pregnant or postpartum	8	6	8	1	0	0
Drug addiction	13	12	13	3	2	0
Serum monoclonal gammopathy	2	2	2	1	0	0
Miscellaneous (burns, balanitis, rash, unspecified kidney or heart disease, nasal problem, painful urination)	7	5	4	0	0	0
No symptoms or illness	41	30	37	2	0	2
% False positives		80	91.3	12.5	2.5	5.0

though the former study also showed a very large percentage (56%) of borderline results in a healthy control group. Taken together, all these findings suggest that the FTA-ABS could be a source of confusion for a physician who initially suspected that the patient had a herpetic infection.

Although as many as one-half of the patients in the BFP group had no obvious symptoms or illness, most of them with symptoms had moderately severe or even fatal diseases or conditions, including other venereal diseases, heroin addiction, autoimmune diseases, carcinoma, and myeloma. These results emphasize the fact that patients with apparent BFP reactions and no obvious symptoms need to be evaluated carefully for underlying illnesses, as suggested by others (6, 8).

Many studies during the 1950s and 1960s (1, 7, 11) showed that false-positive reagin tests were associated with a multitude of illnesses or conditions other than syphilis. More recent studies have indicated that the FTA-ABS sometimes gives a false-positive reaction in cases of systemic lupus erythematosis, rheumatoid arthritis, other diseases associated with abnormal types or amounts of immunoglobulins (4, 5), herpes infections (10), and even pregnancy (3). Our findings illustrate that in addition to the latter diseases and conditions, false-positive or borderline FTA-ABS results can also be associated with a wide variety of viral infections.

Undoubtedly, the most notable finding of this study is that the HATTS test gives less than half as many false-positive reactions as the FTA-ABS. Another obvious advantage of the HATTS over the FTA-ABS is a more clear-cut reaction (i.e., either positive or negative) with no "borderline" results. This is very significant since as many as 11% of sera from nonsyphilitic patients with infectious diseases showed borderline reactions. Studies we reported previously (Wentworth et al., in press) have demonstrated that the HATTS test is almost as sensitive a test for syphilis as the FTA-ABS, since agreement between HATTS and FTA testing was found to be over 93%.

In view of our findings and those of others, we conclude that the HATTS test is preferable to the FTA-ABS test for routine confirmation of a positive RPR or VDRL. In addition, HATTS is less expensive and much easier to perform than FTA-ABS.

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