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Evaluation of the Fluorescent Treponemal Antibody-Absorption (FTA-Abs) Test Specificity

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Serum samples from 43 patients with positive test for syphilis only in the FTA-Abs test, were evaluated. Three had primary or treated syphilis. Twenty-one (49%) had clinical and/or serological signs of Lyme borreliosis as assessed by whole-cell sonicate *Borrelia burgdorferi* ELISA and Western blot techniques. Seven (16%) had genital *Herpes simplex* infection and the remaining 12 patients, miscellaneous disorders. In control sera from 30 patients with Lyme borreliosis an isolated positive FTA-Abs reaction was found in 13 patients (43%). Elevated Borrelia ELISA titres were found in nine of 30 (30%) syphilitic patient serum samples, whereas Western blots for Borrelia were negative. Six per cent of healthy blood donors were seropositive for Borrelia. Lyme borreliosis is an important cause of cross-reactions in the FTA-Abs test. Other serological tests for syphilis and Western blot for Borrelia are useful for discrimination. *Key words: Cross-reaction; Syphilis; Lyme borreliosis.*

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The fluorescent treponemal antibody-absorption (FTA-Abs) test is the first of the established serological tests for syphilis to become reactive in the early stage of the disease, usually, around the time of the appearance of the chancre (1), followed 1–2 weeks later by the rest of the standard tests. The current recommendation (2) is to use the FTA-Abs test as a confirmatory test for the serological diagnosis of syphilis. However, for the diagnosis of late syphilis, the use of a treponemal test (i.e. FTA-Abs and Treponema pallidum haemagglutination assay, TPHA) is mandatory, since non-treponemal tests can prove non-reactive in up to 30% (3,4). Although the FTA-Abs test has an overall high specificity, false-positive reactions have been reported in a variety of acute and chronic diseases, e.g. systemic lupus erythematosus, rheumatoid arthritis, genital herpes simplex, and diabetes mellitus (5–9). Cross-reactivity with the spirochete *Borrelia burgdorferi*, the causative agent of Lyme borreliosis, has previously been described (10–14).

The aims of this study were three-fold, firstly to evaluate patient sera with an isolated positive FTA-Abs test, secondly to study sera from patients with Lyme borreliosis with respect to FTA-Abs results, and thirdly to investigate sera from patients with syphilis with respect to reactivity in Borrelia serology.

PATIENTS AND METHODS

Patients and controls

During a 2-year period serum samples from 43 patients examined for syphilis serology evidenced an isolated positive FTA-Abs test result, i.e. sera proved negative in the WR (Wassermann reaction), VDRL (Venereal Disease Research Laboratory) and TPHA tests. Thirteen of the patients were women, median age 38 years (range 11–74) and 30 were men, median age 41 years (range 23–78). The patients were identified retrospectively and the cases were evaluated by reviewing the of medical records (40 of 43 were available).

Sera from three different groups served as controls: 1) 30 patients with known Lyme borreliosis, 20 women, median age 55 years (range 5–85) and 10 men, median age 42 years (range 12–79); 2) 30 patients with different stages of syphilis, 5 women, median age 38 years (range 21–48) and 25 men, median age 36 years (range 17–77); 3) 100 healthy blood donors, 45 women and 55 men, median age 39 (range 26–63) and 41 years (range 20–65), respectively.

Serological methods for syphilis

All sera were investigated in the FTA-Abs test according to a modification of the original method (15). Commercial preparations of *Treponema pallidum* antigen and sorbent (extract of Reiter treponemas) (Bio Mérieux, Marey-l'Etoile, France) and fluorescein isothiocyanate (FITC)-labelled sheep anti-human immunoglobulin (gamma and light chains) (National Bacteriological Laboratory (NBL), croscope equipped for incident UV-light. All FTA-Abs

Table I. *Borrelia ELISA titres and Western blot in patients with isolated positive FTA-Abs test and clinical and/or serological evidence of Lyme borreliosis.*

Sex/Age	FTA-Abs titre	Borrelia				Clin. signs of borreliosis
		ELISA titre		Western blot		
		IgG	IgM	IgG	IgM	
M/54	1/320	540	630	+	+	Facial nerve palsy
F/38	1/320	360	170	–	–	Neuroborreliosis
M/44	1/640	750	110	–	–	Arthritis, ECM history
M/71	1/640	4 500	600	+	–	Meningitis
F/55	1/640	>2 000	1 330	+	–	Arthritis, ECM history
F/74	1/640	3 700	120	+	+	Meningitis
F/43	1/1 280	1 380	170	+	–	Myalgia, arthralgia, ECM history
M/54	1/2 560	1 530	210	+	–	ACA
F/11	1/5 120	840	140	–	–	LABC
M/60	1/10 240	1 130	340	+	–	Myalgia, arthralgia
F/31	1/20	150	210	+	+	No clinical signs
M/65	1/20	410	<100	+	–	"
F/59	1/160	1 240	<100	+	–	"
M/52	1/160	1 100	130	+	–	"
F/25	1/640	5 200	970	+	–	"
M/47	1/1 280	4 300	200	+	–	"
M/41	1/1 280	1 570	430	+	+	"
M/39	1/1 280	800	<100	+	–	"
M/26	1/2 560	1 170	260	+	–	"
M/39	1/2 560	720	140	+	–	"
M/23	1/5 120	1 500	470	+	–	"

F: female, M: male, ECM: erythema chronicum migrans, ACA: acrodermatitis chronica atrophicans, LABC: lymphadenosis benigna cutis.

tests were examined by one of us (B.C.). End-point titres of two-fold dilutions were recorded. FTA-Abs-positive sera were analysed with the WR and VDRL tests (NBL, Stockholm) according to standard methods and the TPHA test (Fujirebio Inc., Tokyo, Japan) following the manufacturer's instructions.

Preparation of Borrelia antigen

Spirochetes from the Swedish *Borrelia burgdorferi* ACA-1 strain, a skin isolate from a patient with acrodermatitis chronica atrophicans, were grown for 5 days at 32°C in BSK medium (16). Briefly, the cells were harvested by centrifugation and washed in phosphate-buffered saline. The pellet was resuspended and sonicated and the supernatant containing the soluble antigen was used. The same antigen was used for the ELISA test and the Western blot (17).

Enzyme-linked immunosorbent assays for Lyme borreliosis

The enzyme-linked immunosorbent assays (ELISA) for Borrelia were performed using a modification of the method described by Engvall & Perlmann (18). Irradiated microtitre plates (Immunolon II, Dynatech Laboratories Inc., Alexandria, Va, USA) coated with aliquots of 100 µl whole-cell sonicated *B. burgdorferi* antigen dissolved in coating buffer (phosphate-buffered saline (PBS) pH 7.4 containing 0.02% NaN_3), and incubated overnight at room temperature and then kept at +4°C until usage. After washing the plates, 100-µl aliquots of patient sera diluted 1/1,000 (or, if appropriate, 1/5,000 and 1/10,000) in PBS with 0.05% Tween 20 were added to the wells. The plates were incubated at room temperature, 1 h for IgG antibody detection and 2 h for IgM antibody detection. After washings, the microtitre plates were incubated overnight at room temperature with aliquots of either a swine anti-human IgG (Orion Diagnostica, Helsinki, Finland) or a goat anti-human IgM (Sigma Chemical Co., St Louis, Mo, USA) alkaline phosphatase conjugate. Finally, the plates were washed and 100 µl substrate was added (*p*-nitrophenyl phosphate (Sigma) dissolved in diethanolamine/water, pH 9.8). The plates were washed three, four and five times, respectively, with PBS containing 0.05% Tween 20.

Automatic reading of the optical density at 405 nm was performed in a Titertek Multiscan (Flow Laboratories, Irvine, Scotland). Positive and negative controls were included in each test and the time for substrate incubation was adjusted to these controls. The ELISA titre was defined as the 405 nm absorbance value multiplied by the serum dilution factor. Each sample was tested in duplicate and the mean value calculated. The upper limit of normal

Table II. *Comparison of FTA-Abs titre and Borrelia Western blot in sera from patients with Lyme borreliosis and primary (P), secondary (S), latent (L) and treated (T) syphilis respectively. Female (F) and male (M)*

Sex/Age	FTA-Abs titre	Borrelia				Lyme	Syphilis
		ELISA titre		Western blot			
		IgG	IgM	IgG	IgM		
F/15	1/40	820	700	+	−	+	
F/81	1/80	920	<100	+	−	+	
M/24	1/320	580	590	−	+	+	
M/12	1/640	4200	1470	+	+	+	
F/66	1/1280	740	1140	+	+	+	
M/68	1/1280	3600	<100	+	−	+	
F/50	1/2560	4200	860	+	+	+	
M/68	1/2560	15000	2700	+	+	+	
F/69	1/2560	3900	590	+	+	+	
F/85	1/5120	1580	710	+	−	+	
M/79	1/10240	14000	1760	+	+	+	
F/72	1/10240	1520	520	+	−	+	
F/78	1/≥81920	6420	2040	+	+	+	
M/34	1/2560	1460	320	−	−		P
M/36	1/5120	780	150	−	−		P
M/38	1/20480	1430	620	−	−		P
M/49	1/5120	570	360	−	−		P/S
F/21	1/40960	1460	220	−	−		P/S
M/38	1/1280	900	230	−	−		L
M/35	1/2560	810	240	−	−		L
M/31	1/10240	1410	130	−	−		L
F/38	1/10240	550	320	−	−		T

values (cut-off) was defined as the 96th percentile of titres in 200 healthy blood donors and outpatients, corresponding to a titre of 450 for IgG and 700 for IgM.

Western blot (Wb) for Lyme borreliosis

The protein content of the antigen was determined by Biorad protein assay (Bio-Rad, Richmond, Calif., USA). The antigen was diluted with an equal volume of sample buffer (0.125 M Tris-HCl buffer, pH 6.8 containing 20% glycerol, 4% sodium dodecyl sulphate (SDS), 0.2 M dithiothreitol and 0.003% bromphenol blue) and boiled on a waterbath for 5 min, before being subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) *ad modum* Laemmli (19). 330 µg protein was separated in 12% polyacrylamide (0.75 mm thick, 16 cm wide). Then the protein bands were transferred to a nitrocellulose sheet (0.45 µm, Bio-Rad) by semi-dry blotting using 1.5 mA/cm^2 for 50 min. The sheet was cut into strips which were blocked by washing in PBS (10 mM Na-phosphate, 0.15 M NaCl, pH 7.4) containing 5% fat-free milk for one hour. The blocked strips were incubated overnight at +4°C with patient sera diluted 1/50 in blocking buffer. After washings with 10 mM Na-phosphate, 0.5 M NaCl, 0.3% Tween 20, pH 7.4, the strips were incubated with alkaline phosphatase conjugated rabbit anti-human IgG (Dakopatts, Copenhagen, Denmark) or goat anti-human IgM (Sigma). The immunocomplexes were detected by reaction with α-naphthyl-phosphate (Sigma) and Fast Red TR-salt (Sigma) in 5 mM Tris buffer pH 8.6 containing 2 mM $MgCl_2$ for 30 min. A low molecular weight standard 14–97 kD (Bio-Rad) was used to identify the Western blot bands.

An immunoblot was considered positive if a band corresponding to the 41 kD flagellum protein was present and if, for IgG two and for IgM one of the low-molecular protein bands corresponding to 20, 22 and 24 kD were also present. All immunoblots were coded and interpreted independently by two of us (A. B. and H.-S. H.) according to the above-mentioned preset criteria without previous knowledge of ELISA titres.

RESULTS

Patients with isolated positive FTA-Abs test

Three of the 43 patients with reactivity only in the FTA-Abs test had clinically diagnosed syphilis. Two of these, both with FTA-Abs titres of 1/80, had primary syphilis. One patient was partially treated, the other showed seroconversion in the WR, VDRL and

TPHA tests in a blood sample taken 10 days later and the FTA-Abs titre was at that time 1/640. ELISA and Western blot for Borrelia proved negative in both cases. The third patient with an FTA -Abs titre of 1/160, had been treated for syphilis 13 years earlier. He had a moderately elevated Borrelia ELISA IgG but Wb was negative. Still another patient had been given tetracycline for treatment of an indolent genital ulcer with inguinal lymphadenitis after a prostitute contact in Thailand, shortly before syphilis testing.

Twenty-one of the 43 patients (49%) had serological signs of Borrelia infection and 10 of these also had clinically suspected borreliosis (Table I). One of these patients, a 38-year-old woman with neuroborreliosis, had Borrelia titres below cut-off (IgG 360 and IgM 170), but later showed a seroconversion.

A clinical diagnosis of genital *Herpes simplex* infection was established for 7 of the 43 patients. The FTA-Abs titres ranged from 1/10–1/640 and the serological tests for Borrelia were negative. Four of these *Herpes simplex* virus (HSV) infections were verified serologically, two being primary and two recurrent. In one further patient, HSV type 2 was demonstrated by immunofluorescence technique. The 2 remaining patients had a history of recurrent herpetic genital lesions.

Eleven of 43 patients with miscellaneous disorders showed FTA-Abs titres between 1/10 and 1/320. All proved negative for Borrelia in both the ELISA test and Wb. Among these, one patient had polymyalgia rheumatica (antinuclear antibody and rheumatoid factor positive), one had type 1 diabetes mellitus and another was an intravenous drug addict (amphetamine) with elevated levels of serum transminases and a history of non-A, non-B hepatitis.

Control sera from patients with Lyme borreliosis

Sera from 30 patients with Lyme borreliosis in different stages were all Wb-positive and had elevated Borrelia titres, either isolated IgG or in combination with IgM. 13/30 (43%) had a positive FTA-Abs reaction (Table II), while the WR, VDRL and TPHA tests were negative.

Control sera from patients with syphilis

Sera from 30 patients in different stages of syphilis also served as controls. The FTA-Abs and TPHA tests proved positive in all cases. Nine of these 30 (30%) patients had elevated Borrelia IgG titres, but Wbs were negative (Table II).

Control sera from healthy blood donors

One of the 100 sera from healthy blood donors showed a weakly positive reaction in the FTA-Abs test at a serum dilution of 1/5, but the WR, VDRL and TPHA tests proved negative. This serum had Borrelia titres below cut-off and Wb was negative.

Six sera (6%) showed serological signs of Borrelia infection, with positive Wbs. Three of these had correspondingly elevated Borrelia titres in ELISA. Two other sera had slightly elevated IgG ELISA titres, but were negative in Wb.

DISCUSSION

In the present study of serum samples from 43 patients with positive test for syphilis only in the FTA-Abs test, 3 were diagnosed as having syphilis. It cannot be excluded that the early antibiotic treatment of one additional patient, with suspected early syphilis, might have masked the development of subsequent serological reactions.

A high rate (49%) of serologically evident Borrelia infections was observed, which reflects the endemic situation of the Stockholm area. Thus it was demonstrated that in the high endemic area of the Stockholm archipelago up to 26% of the inhabitants were seropositive, as compared with 2% in a non-endemic area (20). We observed no correlation between FTA-Abs and the corresponding Borrelia titres. In sera from 16 of these patients high FTA-Abs titres were seen. By contrast, Hunter et al. (13) detected only a very low FTA-Abs titre in patients with Lyme borreliosis.

Genital herpes has been reported to cause false-positive FTA-Abs reactions (8). It was suggested that *Herpes simplex*, being a DNA virus, induced formation of DNA antibodies in the infected host which were incompletely absorbed by the commercially prepared sorbent. These data were not confirmed by Chapel et al. (21) who used another sorbent in their assays. In our study, 16% of the patients had primary or recurrent genital herpes simplex. However, it cannot be ruled out that the sorbent used in our test interfered with the accuracy of these results.

Among the remaining 11 patients, a few had diseases that *per se* have been reported to give non-specific FTA-Abs reactions. Jokinen et al. (7) and later Kraus et al. (6) demonstrated that sera with antinuclear antibodies gave false-positive reactions. Likewise, diabetes mellitus type 1 (9) and drug ad-

diction (22) have been suggested to interfere with the FTA-Abs test.

Of controls with Lyme borreliosis, 43% proved FTA-Abs positive. A wide range of FTA-Abs titres was observed, but these were not correlated to the corresponding Borrelia IgG titres. None of the patients tested was positive in any of the specific or non-specific syphilis tests, as was previously shown by others (11–13).

In our study, 30% (9/30) of controls in different stages of syphilis were found to have Borrelia antibodies in ELISA; none however, had Borrelia antibodies in Western blot. This tallies with previous reports that serum from patients with syphilis cross-reacts with Borrelia (10,11,14), showing an increase in antibody titre to the whole-cell sonicate antigen.

Six of the 100 healthy blood donors studied proved positive in Borrelia Western blot, although only 3 had an antibody increase above the established cut-off in ELISA. It is well known (23) that Western blot is more sensitive than ELISA in detecting Lyme borreliosis. One of the blood donors showed a weak FTA-Abs reaction, but none of the other syphilis tests was positive. No concomitant underlying disease could be verified and a false-positive reaction cannot be excluded.

Insufficient absorption of group-specific antibodies elicited by non-pathogenic saprophytic treponemes may cause low-grade false-positive reactions in the FTA-Abs test (24). It has earlier been shown (25) that the sonicate of Reiter treponemes is more effective in blocking the binding of group-reactive antibodies to *Treponema pallidum* than is the sorbent. This is probably due to the fact that the sonicate contains all soluble antigens of the Reiter treponemes, whereas heat-labile antigens would be destroyed during the preparation of the sorbent.

In conclusion, we have demonstrated that Borrelia infections are an important cause of cross-reactions in the FTA-Abs test. Likewise, elevated Borrelia titres may be expected in sera from patients with syphilis. Other serological syphilis tests and Western blot for Borrelia are therefore important for discrimination.

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REFERENCES

1. Duncan WC, Knox JM, Wende RD. The FTA-Abs test in darkfield positive primary syphilis. JAMA 1974; 228: 859–860.
2. Larsen SA, Farshy CE, Pender BJ, Adams MR, Pettit DE, Hambie EA. Staining intensities in the fluorescent treponemal antibody-absorption (FTA-Abs) test: association with the diagnosis of syphilis. Sex Transm Dis 1986; 13: 221–227.
3. Hart G. Syphilis tests in diagnostic and therapeutic decision making. Ann Intern Med 1986; 104: 368–376.
4. Luger A. Serological diagnosis of syphilis: current methods. In: Young H, McMillan A, eds. Immunological diagnosis of sexually transmitted diseases. New York: Marcel Dekker, 1987: 249–274.
5. Kraus SJ, Haserick JR, Lantz MA. Fluorescent treponemal antibody-absorption test reactions in lupus erythematosus. N Engl J Med 1970; 282: 1287–1290.
6. Kraus SJ, Haserick JR, Logan LC, Bullard JC. Atypical fluorescence in the fluorescent treponemal antibody-absorption (FTA-ABS) test related to deoxyribonucleic acid (DNA) antibodies. J Immunol 1971; 106: 1665–1669.
7. Jokinen EJ, Lassus A, Linder E. Fluorescent treponemal antibody (FTA) reaction in sera with antinuclear factors. Ann Clin Res 1969; 1: 77–80.
8. Wright JT, Cremer AW, Ridgway GL. False positive FTA-ABS results in patients with genital herpes. Br J Ven Dis 1975; 51: 329–330.
9. Hughes MK, Fusillo MH, Roberson BS. Positive fluorescent treponemal antibody reactions in diabetes. Appl Microbiol 1970; 19: 425–428.
10. Craft JE, Grodzicki RL, Steere AC. Antibody response in Lyme disease: evaluation of diagnostic tests. J Infect Dis 1984; 149: 789–795.
11. Russel H, Sampson JS, Schmid GP, Wilkinson HW, Plikaytis B. Enzyme-linked immunosorbent assay and indirect immunofluorescence assay for Lyme disease. J Infect Dis 1984; 149: 465–470.
12. Åsbrink E, Hovmark A, Hederstedt B. The spirochetal etiology of acrodermatitis chronica atrophicans Herxheimer. Acta Derm Venereol (Stockh) 1984; 64: 506–512.
13. Hunter EF, Russel H, Farshy CE, Sampson JS, Larsen SA. Evaluation of sera from patients with Lyme disease in the fluorescent treponemal antibody-absorption test for syphilis. Sex Transm Dis 1986; 13: 232–236.
14. Magnarelli LA, Anderson JF, Johnson RC. Cross-reactivity in serological tests for Lyme disease and other spirochetal infections. J Infect Dis 1987; 156: 183–188.
15. Hunter EF, Deacon WE, Meyer PE. An improved FTA test for syphilis, the absorption procedure (FTA-ABS). Public Health Rep 1964; 79: 410–412.
16. Barbour AG. Isolation and cultivation of Lyme disease spirochetes. Yale J Biol Med 1984; 57: 521–525.
17. Stiernstedt GT, Granström M, Hederstedt B, Sköldenberg B. Diagnosis of spirochetal meningitis by enzyme-linked immunosorbent assay and indirect immunofluo-

rescence assay in serum and cerebrospinal fluid. J Clin Microbiol 1985; 21: 819–825.
18. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay ELISA. III. Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen-coated tubes. J Immunol 1972; 109: 129–135.
19. Laemmli UK. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature 1970; 227: 680–685.
20. Gustafson R, Svenungsson B, Gardulf A, Stiernstedt G, Forsgren M. Prevalence of tick-borne encephalitis and Lyme borreliosis in a defined Swedish population. Scand J Infect Dis 1990; 22: 297–306.
21. Chapel T, Jeffries CD, Brown WJ, Stewart JA. Influence of genital herpes on results of fluorescent treponemal antibody absorption test. Br J Ven Dis 1978; 54: 299–302.
22. Feitelson PJ, Junkin AB. A false positive FTA-ABS test in a heroin addict. California Medicine 1973; 119: 73–74.
23. Karlsson M, Möllegård I, Stiernstedt G, Wretlind B. Comparison of Western blot and enzyme-linked immunosorbent assay for diagnosis of Lyme disease. Eur J Clin Microbiol Infect Dis 1989; 8: 871–877.
24. Tringali GR, Cox PM. Reactivity in the FTA-ABS test of rabbits hyperimmunized with nonpathogenic treponemes. Br J Ven Dis 1970; 46: 313–317.
25. Wilkinson AE, Wiseman CC. The role of sorbent in the absorbed fluorescent treponemal antibody (FTA-ABS) test. Proc Roy Med 1971; 64: 422–425.