Study of fluorescent treponemal antibody test on cerebrospinal fluid using monospecific anti-immunoglobulin conjugates IgG, IgM, and IgA*

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SUMMARY The fluorescent treponemal antibody test for cerebrospinal fluid (FTA-CSF) using monospecific conjugates anti-IgG, IgM, and IgA was used to determine the presence of anti-treponemal antibodies in the spinal fluid of 335 patients with primary, secondary, and latent syphilis and symptomatic and asymptomatic neurosyphilis and of patients with certain neurological disorders. Of these, 230 (68.65%) patients had non-reactive results to this test. Of the remaining 105 patients, 78, 63, and 10 had reactive results with anti-IgG, IgM, and IgA conjugates respectively. Of the 129 cases of known syphilis, 11 were diagnosed as primary, 32 as secondary, and 50 as latent, and 36 patients had neurosyphilis. None of the specimens from the patients with primary syphilis gave reactive results to the test. Specimens from 21 (65.62%) of the 32 patients with secondary syphilis, 30 (60%) of the 50 patients with latent syphilis, and all (97.22%), except one, of the 36 patients with neurosyphilis gave reactive results to one at least of the IgG, IgM, or IgA FTA-CSF tests. Among the specimens from patients with secondary syphilis twice as many gave reactive results with anti-IgG conjugate than with anti-IgM conjugate. However, with specimens from patients with latent syphilis and neurosyphilis this ratio was diminished to 1.5:1. The Kolmer complement-fixation test, although superior in sensitivity and specificity to the Venereal Disease Research Laboratory (VDRL) test, in patients with secondary and latent syphilis and neurosyphilis, was greatly inferior to the FTA-CSF test. Data indicate that anti-treponemal antibodies can be detected in the spinal fluid even in patients with no neurological symptoms in cases of secondary syphilis and that the FTA-CSF test can be a valuable tool in the early detection of an immunological response to treponemal infection in the spinal fluid.

Introduction

The fluorescent treponemal antibody absorption (FTA-ABS) test developed by Hunter *et al.* (1964) is frequently used as a confirmatory test for detecting anti-treponemal antibodies in the serum. The advantages of this treponemal test compared with other serological tests are its sensitivity and specificity; and it can be used to assess different classes of immunoglobulins during different stages of syphilitic

infection. The FTA-ABS test using IgM carried out on the serum from newborn infants and developed by Scotti and Logan (1968) has been found useful in differentiating active congenital syphilis from passive transfer of maternal antibodies. Atwood and Miller (1970), studying serum samples from 147 patients with different stages of syphilis, have shown that the anti-treponemal antibodies may belong either to IgG, IgM, or IgA class of immunoglobulins and that almost all patients with primary and secondary syphilis, about 50% of those with latent syphilis, and about 75% of those with tertiary syphilis have serum IgM antibodies. These IgM serum antibodies found in patients with primary syphilis before treatment tend to disappear within two years after

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treatment (O'Neil and Nicol, 1972). On the other hand, the antibodies of the IgG class persist for more than 20 years.

Though the antibodies of the class IgG and IgM increase during the early seronegative stage of syphilis, those of the three classes are present only in the stage when most of the standard serological tests for syphilis give reactive results (Menke and Van der Sluis, 1974).

The serum FTA-ABS test was adapted to cerebrospinal fluid (FTA-CSF), to determine anti-treponemal antibodies in spinal fluids, by Duncan *et al.* (1972) and Kasatiya *et al.* (1972), who found the test to be more sensitive than any of the other serological tests available. As the IgM (Schultze and Heremans, 1966) and IgA (Sheth, 1971) immunoglobulins are not normally present in the spinal fluid, and IgG immunoglobulins are present only in very small quantities (Sheth, 1971), the presence of any qualitative or quantitative change of these immunoglobulins detected by the tests of immunodiffusion or indirect fluorescent antibody may indicate the local production of anti-treponemal antibodies in a syphilitic patient or may even indicate an increase in permeability due to damage to the blood-brain barrier.

The present study was undertaken to evaluate the FTA-CSF test, with monospecific anti-IgG, IgM, and IgA conjugates, and was carried out on samples of spinal fluid from patients with and without symptoms referred to two local antivenereal clinics affiliated to the University of Montreal.

Materials and methods

Samples of spinal fluid were collected from 335 patients referred to the venereal diseases clinics of two University hospitals and sent to the State laboratory within 24 hours of collection. The sera from all these patients gave reactive results to the VDRL or FTA-ABS tests or both. Specimens were stored under refrigeration. VDRL and Kolmer complement fixation (CF) tests were carried out as recommended by the US Center for Disease Control (1964). The FTA-CSF test was carried out on all undiluted, unabsorbed spinal fluid specimens according to the method of Duncan *et al.* (1972). The same reagents, except the conjugates, with the same lot numbers supplied by Aerojet General Company (Organon) were used. The Redi-Fix* (Aerojet General Company) slides were stored at -20°C . The fluorescein conjugated monospecific anti-human gammaglobulins IgG (lot no. 2201X001A1), IgM (lot no. 2201X003A1), and IgA (lot no.

2212R002A1) from Hyland Laboratories were used in a dilution of 1/300, 1/70, and 1/40 respectively in order to get the same intensity of fluorescence with reactive (4+), weakly reactive, and negative serum controls. The same serologist examined all the slides with a Leitz Orthoplan microscope equipped with an incident light system according to the method described by Kasatiya *et al.* (1972). BG 12, 5 mm exciter, and OG1 absorption filters were used.

Results

Spinal fluid specimens from 230 (68.65%) of the 335 patients, irrespective of their clinical state, gave non-reactive results to the FTA-CSF test (Table 1). Only the FTA-CSF test using IgG gave reactive results in 9.85%, using IgM in 6.26%, and using IgA in 1.79% of the patients examined. Of the total of 105 patients with reactive results in either of the tests, 78 (74.28%), 63 (60%), and 10 (9.52%) gave reactive results with IgG, IgM, and IgA conjugate respectively.

The reactivity of different tests carried out on the spinal fluid specimens from 129 patients with known syphilis are shown in Table 2. Because the information on the treatment schedules of these syphilitic patients was not available, studies related to the elimination of IgG and IgM antibodies following treatment could not be done. The spinal fluid specimens of only 86 (66.66%) of 129 patients with all stages of syphilis or of 86 (72.88%) of 118 patients with all stages of syphilis except primary gave reactive results with FTA-CSF tests using IgG or IgM or both. The FTA-CSF test using IgG gave reactive results in 60.46%, and the FTA-CSF test using IgM in 39.53% of patients. IgA antibodies were detected in four of the patients with syphilis. Only IgG or IgM antibodies were detected in 19.37% and 6.20% of the patients respectively. VDRL and complement fixation tests gave reactive results in only 17.82% and 27.90% of patients respectively, and there was no patient in whom only the VDRL or CF test gave reactive results while the FTA-CSF test gave non-reactive results. The results of the 129 patients with syphilis were divided into 10 different reactivity patterns based on the five spinal fluid serological tests.

Of the 129 patients with syphilis, 11 were diagnosed as having primary, 32 as secondary, and 50 as latent syphilis, and 36 had neurosyphilis. The specimens from all of the 11 patients with known primary syphilis gave non-reactive results in all five tests. Table 3 shows the reactivity pattern of the results of 32 patients with secondary syphilis. The FTA-CSF test using IgG, the most sensitive of

*Trade name for methanol-prefixed slides containing *Treponema pallidum* cells on a coverglass.

Table 1 Reactivity of FTA-CSF test using monospecific conjugates with cerebrospinal fluid specimens from 335 patients with and without symptoms

Reactivity pattern No.	FTA-CSF test using			Patients	
	IgG	IgM	IgA	No.	%
1	+	+	+	1	0.29
2	+	+	—	41	12.23
3	+	—	—	33	9.85
4	—	+	—	21	6.26
5	—	—	+	6	1.79
6	+	—	+	3	0.89
7	—	—	—	230	68.65
Reactive results					
Total no.	78	63	10	335	
%	23.28	18.80	2.98		

+ Positive

— Negative

Table 2 Reactivity of VDRL, CF, and FTA-CSF tests on specimens of cerebrospinal fluid from 129 patients with known syphilis

Reactivity Pattern no.	Tests		FTA-CSF using			Patients	
	VDRL	CF	IgG	IgM	IgA	No.	%
1	+	+	+	+	+	1	0.77
2	+	+	+	+	—	20	15.50
3	+	+	+	—	—	2	1.55
4	—	+	+	+	—	8	6.20
5	—	+	+	—	—	5	3.87
6	—	—	+	+	—	14	10.85
7	—	—	+	—	+	3	2.32
8	—	—	+	—	—	25	19.37
9	—	—	—	+	—	8	6.20
10	—	—	—	—	—	43*	33.33
Reactive results							
Total no.	23	36	78	51	4	129†	
%	17.82	27.90	60.46	39.53	3.10		

*All 11 patients with primary syphilis are included

†Patients with treated and untreated syphilis are included

+ Positive

— Negative

Table 3 Reactivity of VDRL, CF and FTA-CSF tests on specimens of cerebrospinal fluid from 32 patients with known secondary syphilis*

Reactivity Pattern no.	Tests		FTA-CSF using			Patients	
	VDRL	CF	IgG	IgM	IgA	No.	%
1	+	+	+	+	+	0	0
2	+	+	+	+	—	4	12.50
3	+	+	+	—	—	2	6.25
4	—	+	+	+	—	1	3.12
5	—	+	+	—	—	3	9.37
6	—	—	+	+	—	4	12.50
7	—	—	+	—	+	0	0
8	—	—	+	—	—	6	18.75
9	—	—	—	+	—	1	3.12
10	—	—	—	—	—	11	34.37
Reactive results							
Total no.	6	10	21	10	0	32	
%	18.75	31.25	65.62	31.25	0		

*Diagnosis based on clinical picture and blood serology before lumbar puncture

— Negative

+ Positive

all the tests, gave reactive results in 65.62% of the patients. The VDRL test was the least sensitive, giving reactive results in only 18.75% of the patients.

The clinician may not be able to delineate precisely between early latent, late, treated, and untreated syphilis and asymptomatic neurosyphilis, all of which may give different reactivity and sensitivity patterns. Table 4 shows that in all the different stages of syphilis the VDRL test was the least sensitive in latent syphilis, whereas the FTA-CSF test using IgG remained the most sensitive.

Almost 98% of the 36 patients with known neurosyphilis gave reactive results to the FTA-CSF test using IgG (Table 5). The Kolmer complement fixation test gave reactive results in 63.88% of the patients, which agrees with the previous study done by Kasatiya *et al.* (1972). In the different stages of syphilis, the VDRL test was most sensitive in neurosyphilis, giving reactive results in 44.44% of the specimens of spinal fluids from 36 patients.

Data from the patients without syphilis was scanty. The diagnosis and FTA-CSF results of 172 patients is shown in Table 6; only two, with cerebral tumours, had reactive results.

Table 4 Reactivity of VDRL, CF, and FTA-CSF tests on specimens of cerebrospinal fluid from 50 patients with known latent syphilis

Reactivity Pattern no.	Tests					Patients	
	VDRL	CF	FTA-CSF using			No.	%
			IgG	IgM	IgA		
1	+	+	+	+	+	0	0
2	+	+	+	+	—	1	2.00
3	+	+	+	—	—	0	0
4	—	+	+	+	—	1	2.00
5	—	+	+	—	—	1	2.00
6	—	—	+	+	—	6	12.00
7	—	—	+	—	+	1	2.00
8	—	—	+	—	—	13	26.00
9	—	—	—	+	—	7	14.00
10	—	—	—	—	—	20	40.00
Reactive results							
Total no.	1	3	23	15	1	50	
%	2.00	6.00	46.00	30.00	2.00		

— Negative

+ Positive

Table 5 Reactivity of VDRL, CF, and FTA-CSF tests on specimens of cerebrospinal fluid from 36 patients with known neurosyphilis

Reactivity Pattern no.	Tests					Patients	
	VDRL	CF	FTA-CSF using			No.	%
			IgG	IgM	IgA		
1	+	+	+	+	+	1	2.77
2	+	+	+	+	—	15	41.66
3	+	+	+	—	—	0	0
4	—	+	+	+	—	6	16.66
5	—	+	+	—	—	1	2.77
6	—	—	+	+	—	4	11.11
7	—	—	+	—	+	2	5.55
8	—	—	+	—	—	6	16.66
9	—	—	—	+	—	0	0
10	—	—	—	—	—	1	2.77
Reactive results							
Total no.	16	23	35	26	3	36	
%	44.44	63.88	97.22	72.22	8.33		

— Negative

+ Positive

Table 6 Reactivity of FTA-CSF test on specimens of spinal fluid from patients with diseases other than syphilis

Disease	No. of patients	FTA-CSF test using			Total no. of patients (reactive)
		IgG	IgM	IgA	
Cerebral metastasis	1	—	—	—	0
Cerebral syndrome	1	—	—	—	0
Cerebral thrombosis	7	—	—	—	0
Cerebral tumour	4	—	—	—	0
Cerebral tumour	2	+	+	+	2
Dementia	2	—	—	—	0
Discal hernia	30	—	—	—	0
Encephalitis	11	—	—	—	0
Epilepsy	42	—	—	—	0
Fever	1	—	—	—	0
Hydrocephaly	2	—	—	—	0
Hypertension	1	—	—	—	0
Hyperthermia	1	—	—	—	0
Hypophyseal tumour	3	—	—	—	0
Labyrinthitis	1	—	—	—	0
Meningitis	28	—	—	—	0
Multiple sclerosis	26	—	—	—	0
Neuritis	2	—	—	—	0
Paraplegia	1	—	—	—	0
Pneumonia	1	—	—	—	0
Polyarthrititis	1	—	—	—	0
Toxoplasmosis	1	—	—	—	0
Tuberculosis	1	—	—	—	0
Vasculitis	1	—	—	—	0
Viraemia	1	—	—	—	0
Total	172				2

— Negative

+ Positive

Discussion

The FTA-CSF tests using IgG and IgM seem to be specific and sensitive and together may detect up to 66.66% of previous or recent, treated or untreated cases of syphilis. The superiority of the fluorescent treponemal antibody test regarding sensitivity and specificity to other serological tests has already been reported by Harris *et al.* (1960), Escobar *et al.* (1970), Kasatiya *et al.* (1972), Duncan *et al.* (1972) and Wilkinson (1973).

Abnormal specimens of fluid are found in the primary stage of syphilis in 7–25% of patients. In fully developed untreated secondary syphilis various reports give ranges from 26–78%, with a mean of about 40–45%. In treated and more advanced, untreated secondary syphilis the proportion falls to between 24 and 26% (Stokes *et al.*, 1944). Oxelius *et al.* (1969), using the immunodiffusion test, showed the presence of IgG and IgA in the spinal fluid of patients with secondary syphilis and of IgM in untreated patients with neurosyphilis. Their results were confirmed by Bowszyc (1975), who using the FTA-CSF test found IgM and probably IgA in early, late latent, and late symptomatic and asymptomatic cases of syphilis. Our data support the observations of the above authors that the FTA-CSF test using IgG is the most sensitive and

that the IgG antibodies can be detected in the spinal fluid of patients with different stages of syphilis except the primary. The reactivity obtained in the FTA-CSF test using IgA may be either a syphilitic response, an indication of bacterial, viral, and immunological diseases, or even an association of the two. Sheth (1971) found IgA antibodies in an immunodiffusion test on the spinal fluid of patients with bacterial meningitis. On the other hand, Duncan and Kuhn (1973) observed a reactivity with IgG conjugate but did not find any reactivity with IgM conjugate in the FTA-CSF test performed on specimens of cerebrospinal fluid from 29 patients with neurosyphilis. Our data do not agree completely with those of Duncan and Kuhn (1973), as we obtained reactivity in the FTA-CSF test using IgM on specimens of spinal fluid from patients with all stages of syphilis except the primary.

The relatively low percentage of reactivity for the FTA-CSF test using IgM in secondary syphilis (Table 2) may be either because most of these specimens come from treated patients where IgM antibodies tend to disappear after treatment, or because a high concentration of IgG antibodies in undiluted spinal fluid probably masked the reaction of IgM antibodies, or because there might be damage to blood-brain barrier (especially in

secondary syphilis) which permitted an increased flow of IgG antibodies from serum to spinal fluid, or because the present techniques are too insensitive to detect the small quantities of IgM antibodies present in the very early stages of syphilis or in treated syphilis (Nerenberg and Prasad, 1975).

Though the FTA-CSF test using IgM can be valuable in the assessment after treatment because of the disappearance of IgM antibodies its sensitivity in the diagnosis of syphilis is yet to be evaluated.

On the other hand, the FTA-CSF test using IgG, which seems to be a highly sensitive test, cannot differentiate between previous and currently active syphilis. The reactivity of fluorescent antibody tests on the spinal fluid of asymptomatic and treated patients raises doubts about the efficacy of present treatment. As early as 1962, Collart *et al.* showed the presence of treponemal forms resembling *Treponema pallidum* in the lymph nodes of patients treated with penicillin. Tramont (1976) found infective, viable treponemes in specimens of spinal fluid (which gave reactive results to the FTA-ABS test) from neurologically asymptomatic patients with non-reactive results to the VDRL test. Initial treatment with low recommended doses of intramuscular penicillin did not alter the spinal fluid with regard to its reactivity in FTA-ABS test or its ability to infect rabbits.

In view of these findings of FTA-CSF values in syphilitic patients the clinical classification of syphilis and the therapeutic approach may need be re-evaluated.

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