Acta Dermatovener (Stockholm) 52: 71–74, 1972

EVALUATION OF THE C.S.F. F.T.A. ABS TEST IN LATENT AND TERTIARY TREATED SYPHILIS

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Abstract. Specific test for T. pallidum antibodies are briefly discussed. The cerebrospinal fluid parameters (viz. W.R. Lange curve, protein content, cell count and F.T.A. ABS) are considered in 27 patients with treated latent and tertiary syphilis and related to any clinical evidence of neurosyphilis. It was found that the C.S.F. F.T.A. ABS is better than the other parameters as an indicator of neurosyphilis, being positive in 75% of cases with clinical evidence of neurosyphilis but nevertheless negative in a proportion of cases when there is no clinical doubt of the presence of syphilitic C.N.S. involvement. Since the serological F.T.A. ABS was positive in all 27 patients, it is concluded that this is a more sensitive test of all forms of syphilis, including neurosyphilis, in this group of patients. It does not, however, provide a *specific* test for neurosyphilis. A selective review of the literature of C.S.F. tests in syphilis is carried out. The C.S.F., T.P.I., F.T.A. 200 and F.T.A. ABS are all apparently less sensitive than the serological F.T.A. ABS. The original F.T.A. $^1/_5$ test carried out on the C.S.F. however, appears to be a much more sensitive indicator of neurosyphilis than any other C.S.F. tests though its specificity is not known.

The first specific test for syphilis was introduced by Nelson & Mayer (11) in 1949 when the capacity of a test serum to immobilise a rabbit-adapted strain of T. pallidum indicated the presence of specific immobilising antibodies separate and distinct from "reagin" (Treponemal Immobilisation Test or T.P.I.).

The inconvenience of maintaining a population of syphilitic rabbits as a source of antigen for the T.P.I. test led to a search for a reliable test which would employ killed T. pallidum as antigen. The fluorescent treponemal antibody test (F.T.A.) which includes this principle has undergone progressive modification to increase its specificity since its introduction by Deacon et al. (3) in 1957. Nowadays the F.T.A. ABS test is carried out in many larger hospitals. In early syphilis this test is more sensitive than the reagin tests which in turn are more sensitive than the T.P.I. Its sensitivity in late syphilis is at least equal to that of the T.P.I. though it is probably somewhat less specific and may occasionally give rise to false positive reactions particularly in lupus erythematosis (10). The great advantage of the F.T.A. ABS is that it can be performed with commercially available reagents in any hospital with suitably trained technical staff and fluorescent microscopic facilities.

MATERIALS AND METHODS

The more important steps in the F.T.A. ABS test as carried out in the Royal Victoria Hospital, Belfast, are as follows:

1. The test serum (or C.S.F.) is diluted 1.5 in a sonicate of Reiter treponemes ("Sorbent").
2. This is then applied to previously prepared slides of killed T. pallidum (Baltimore Biological Laboratories). These slides can be stored for long periods at −20°C. The serum-sorbent preparation is kept on the slide for 30 min at room temperature (experience has shown that there is no advantage to be gained from keeping experimental conditions at exactly 37°C).
3. The slide is then washed with a solution of phosphate-buffered saline (P.B.S.), excess moisture is drained off and the slide is left to dry out at room temperature.
4. A conjugate of fluorescein isothiocyanate (Baltimore Biological Laboratories) and anti-human gamma immunoglobulin (Hyland) is then added to the slide for a further 30 min after which the slide is once again washed with P.B.S.
5. The slide is now mounted using a buffered glycerol mounting medium (Difco) and then examined microscopically for fluorescence.

Commercially available conjugate has not been found completely reliable in this laboratory and it has been

Table I. *Results*

	No. of patients	Positive clinical signs of neurosyphilis	No clinical signs of neurosyphilis
Positive C.S.F. F.T.A. ABS	15	15	0
Negative C.S.F. F.T.A. ABS	12	5	7

Table II. *Cells < 5 c/mm*

C.S.F. F.T.A. ABS positive				C.S.F. F.T.A. ABS negative			
Total	Cells	Protein	C.N.S. signs	Total	Cells	Protein	C.N.S. signs
1	7	31	+	3	8	84	–
					6	31	–
					230	35	+

found preferable to prepare the conjugate ourselves. The technique is felt to be of importance and will be briefly outlined. The fluorescein isothiocyanate (F.I.T.C.) is added very slowly to the anti-human goat immunoglobulin over a period of 15 min at a temperature of 4°C. It has been found important to mix the reagents very gently. The conjugate is then transferred to a mechanical mixer where it is gently agitated at 4°C for a further 24 hours. Before this operation all preservative has been removed from the anti-human immunoglobulins by dialysis with P.B.S.

Control tests are performed on each new batch of conjugate using known positive sera in order to achieve a uniform standard of fluorescence. Samples of known positive sera which give weak fluorescence are kept for carrying out control tests in "doubtful" cases. An important source of error in the test has been found to be decay of the ultra-violet light source and we stress that a record be kept of the hours this has been in use, and that it be changed as recommended by the manufacturers.

Scope and objects of the investigations

The group under consideration was comprised of patients with latent or tertiary treated syphilis attending the hospital for routine surveillance between May and October 1970. In 27 instances it was felt that C.S.F. investigations were indicated and in addition to the usual parameters, viz: cell count, protein content, W.R. and Lange curve, it was decided to carry out the F.T.A. ABS test on the C.S.F. in these cases.

Our objects in carrying out these tests were as follows:

I. To determine any correlation that might exist between clinical evidence of neurosyphilis and the C.S.F. F.T.A. ABS reaction in the group of patients (Table I).

II. To determine if there were any correlation between the C.S.F. F.T.A. ABS and a cell count greater than 5 c/mm and a protein content greater than 50 mg% (Tables II and III).

III. To see if the results might indicate any correlation between raised cell counts and protein in the C.S.F. and clinical signs of neurosyphilis in this group of patients (Tables II and III).

The criteria adopted to satisfy "clinical signs of neurosyphilis" were as follows:

(i) Argyll-Robertson type pupils plus evidence of posterior column sensation impairment as demonstrable by loss of ankle jerks and/or diminution or absence of vibration and muscle-joint sense.

(ii) A clear history of "G.P.I."—which usually dated back to the first contact the patient had with this clinic. In all cases there was serological evidence to support this diagnosis together with a history of abnormal C.S.F. finding.

RESULTS

Correlation between C.S.F. F.T.A. ABS and clinical evidence of neurosyphilis

We emphasize that the serological F.T.A. ABS was positive in all 27 patients and also that the C.S.F., W.R. and Lange curves had reverted to normal in all cases.

Table I shows that the C.S.F. F.T.A. ABS was positive in 15 of the 27 patients. We considered that in all 15 patients there was satisfactory clinical evidence of neurosyphilis and further that in the 12 patients who were C.S.F. F.T.A. ABS negative, 5 showed good clinical evidence of neurosyphilis.

Hence a positive C.S.F. F.T.A. ABS was found in only 15 out of 20 (75%) patients with clinical evidence of neurosyphilis. In no instance, however, did we find a positive C.S.F. F.T.A. ABS when

Table III. *Protein > 50 mg%*

Upper limit of normal cells is taken to be 5 c/mm. Upper limit of normal protein is taken to be 50 mg%

C.S.F. F.T.A. ABS positive				C.S.F. F.T.A. ABS negative			
Total	Protein	Cells	C.N.S. signs	Total	Protein	Cells	C.N.S. signs
4	65	< 1	+	2	84	8	+
	58	< 1	+		58	< 3	–
	65	< 1	+				
	58	< 1	+				

W.R. negative and Lange curve normal in all 27 cases.

there was no clinical evidence of neurosyphilis. We conclude that this test was sensitive enough to indicate 75 % of cases with C.N.S. involvement in patients with treated latent and tertiary syphilis and gave no "false positive" results.

Correlation between C.S.F. F.T.A. ABS and pleocytosis i.e. < 5 cells/mm^3

Table II shows that 4 of the patients showed a pleocytosis. In one of these the C.S.F. F.T.A. ABS was positive and this case also showed clinical evidence of neurosyphilis. In 3 cases the pleocytosis was related to a negative C.S.F. F.T.A. ABS and in one of these there was clinical evidence of syphilitic C.N.S. involvement.

We conclude that no correlation exists either between C.S.F., F.T.A. ABS findings and pleocytosis, or between pleocytosis and clinical evidence of syphilitic C.N.S. involvement in latent or tertiary treated syphilis.

Correlation between C.S.F. F.T.A. ABS and raised C.S.F. protein i.e. > 50 mg %

Table III shows that there was a raised protein in 6 patients. In 4 of these the C.S.F. F.T.A. ABS was positive and all of these had clinical evidence of C.N.S. involvement. In the 2 patients where a raised protein was associated with negative C.S.F. F.T.A. ABS, one (which also showed a pleocytosis) had positive clinical C.N.S. findings. Thus a positive correlation was demonstrated between raised protein and positive C.N.S. signs in 5 cases and a negative correlation in one. Raised protein in the C.S.F. therefore was present in 5 out of 20 (25 %) of patients with C.N.S. signs as against 15 out of 20 (75 %) of them with positive C.S.F. F.T.A. ABS and in none of the latter was a positive test associated with negative clinical findings in the C.N.S.

We conclude that the C.S.F. F.T.A. ABS is more sensitive and more specific than raised protein as an indicator of C.N.S. involvement in this group of patients.

DISCUSSION

It is probable that C.S.F. investigation in patients with early treated syphilis is an unnecessary procedure. Jefferiss (8) expressed the view that "cerebrospinal fluid is never significantly abnormal when examined after full Penicillin treatment of early syphilis". Fernando (6) added weight to this view when he found that out of 231 patients treated adequately for primary or secondary syphilis "only 3 revealed a very slight (probably insignificant) elevation of the cell count only". In all Fernanlo's cases C.S.F. analysis had been carried out not less than 2 years after completion of initial treatment.

Walshe (14) believed that increased cell count was the first parameter to show abnormality in developing neurosyphilis. Only later, and in the following order, would further abnormal C.S.F. findings become apparent viz. increased total protein, positive W.R. and abnormal Lange curve. The earlier work of Dattner & Thomas (2) supports the view that pleocytosis is the most sensitive indicator, amongst these four parameters, of active neurosyphilis.

The indications for C.S.F. examination in patients who have progressed to established neurosyphilis, however, are different. King & Nichol (9) discussed the relevance of this in the early stages of neurosyphilis and conclude "in the absence of clinical progression and with tests of cerebrospinal fluid which become and remain normal, it is nevertheless a wise precaution to perform further tests of the cerebrospinal fluid after a further two years". Dattner et al. (1) believed that relapse of all types of neurosyphilis "rarely, if ever occurs more than two years after effective antisyphilis treatment".

We would also like to stress the importance of not putting too much reliance on normal values in the C.S.F. with respect to cells, protein, W.R. and Lange curve when investigating a case of possible neurosyphilis. In this connection we refer to the work of Dewhurst (4) who showed that in 58 cases of untreated neurosyphilitic psychosis the C.S.F. showed "a high proportion of patients with low cell counts and total protein levels". His inference was that "untreated" cases in fact contained a large proportion of patients who had received enough inadvertent antibiotic therapy to render the C.S.F. parameters normal. The widespread use of antibiotics in the community must, therefore, be borne in mind when assessing the C.S.F. results in cases of suspected neurosyphilis.

We now consider briefly some of the relevant work by others on specific tests for syphilis performed on cerebrospinal fluid.

The T.P.I. Harris et al. (7) obtained 82 positive F.T.A. tests from the C.S.F. of 369 patients with treated syphilis as against only 26 with positive T.P.I. Although he did not correlate these findings with clinical evidence of neurosyphilis, it would appear that the C.S.F. F.T.A. is a more sensitive test than the C.S.F. T.P.I.

The original F.T.A. $^1/_5$ test appears to be a very sensitive test for C.N.S. syphilis. As long ago as 1961 Vaisman & Hamelin (13) found no negative C.S.F. F.T.A. tests in 53 patients with neurosyphilis. More recently Escobar et al. (5) found the C.S.F. F.T.A. to be positive in 16 out of 18 cases of diagnosed neurosyphilis (89 %) as against 4 out of 18 (22 %) in the C.S.F. F.T.A. ABS which was equal in this series to the sensitivity of the C.S.F. V.D.R.L. The discrepancy between a correlation of only 22 % against our findings of a 75 % correlation between this test and clinical evidence of neurosyphilis might be explained on grounds of differing techniques in performing the C.S.F. F.T.A. ABS or on differing criteria for making a diagnosis of neurosyphilis. Their paper however, does not describe either of these factors.

The F.T.A. 200. Using this test on C.S.F., Neilson & Idsøe (12) found it to be only slightly more sensitive than the T.P.I. and also to be negative in some patients with undoubted neurosyphilis.

CONCLUSION

We conclude that the C.S.F. F.T.A. ABS is a specific but only moderately sensitive test to confirm a clinical diagnosis of neurosyphilis. The serological F.T.A. ABS is very sensitive but cannot be regarded as a specific test for neurosyphilis as it is positive in all forms of syphilis. It may be that the C.S.F. F.T.A. $^1/_5$ may prove to be the best confirmatory test for neurosyphilis, though further investigation will be required to confirm its specificity in this respect.

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Received March 25, 1971

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