Brit. J. vener. Dis. (1976) **52**, 219

IgM-FTA test in syphilis in adults
Its relation to clinical findings

A. E. WILKINSON AND P. RODIN
From the V.D. Reference Laboratory (P.H.L.S.) and the Whitechapel Clinic, The London Hospital

Summary

IgM-FTA tests have been carried out on 209 sera from 169 patients with treated or untreated syphilis at various stages and on 128 sera from 109 patients, born in areas where yaws is or was prevalent, with treated or untreated latent treponemal disease.

IgM anti-treponemal antibody was found in virtually all cases of untreated early or early latent syphilis but in only 23 per cent. of sera from patients with untreated late latent syphilis. After treatment the antibody usually disappeared within one year, but it persisted in a minority of patients, including some treated for late symptomatic or congenital syphilis. Except in isolated cases there was no clinical evidence to suggest continued disease activity, although a third of the patients in whom the antibody persisted for more than 2 years after treatment were noted to be homosexuals.

The test may assist in differentiating untreated early latent from late latent syphilis.

Introduction

In syphilis, as in many other diseases, the early antibody response to infection is the production of immunoglobulin M (IgM), rapidly followed by the appearance of immunoglobulin G (IgG), which soon becomes predominant (Julian, Logan, and Norins, 1969). Other studies have shown that specific anti-treponemal IgM may be present in latent and late syphilis, even after treatment (Atwood and Miller, 1969; 1970; Logan, Norins, Atwood, and Miller, 1969; Julian, Logan, Norins, and Scotti, 1971 ; O'Neill and Nicol, 1972). Atwood and Miller (1969), who found IgM-FTA tests positive in two of five patients with treated late syphilis, suggested that this might reflect persistence of the antigen, although both had been treated only 6 months before the tests were performed. In a detailed study in which serial IgM-FTA tests were carried out on individual patients, O'Neill and Nicol (1972) found that IgM antitreponemal antibody persisted after treatment of primary and secondary syphilis for an average period of 3 to 8 months. They detected this antibody in all of fifteen patients with untreated late infections (latent 5; late symptomatic, 5; congenital, 5). They suggested that arrest of the disease was associated with disappearance of the antibody, and that its persistence might reflect recent or inadequate treatment or re-infection. They considered that the test might give useful information which could assist in the assessment and management of patients. Müller and Loa (1974) agreed with these views.

A common problem in many clinics in Great Britain is posed by immigrants from areas where yaws is, or has been, prevalent and who are found to have positive serological tests for syphilis without any clinical evidence of infection. It is rarely possible to decide with any certainty whether these are due to syphilis or to an old infection with yaws, and such patients are diagnosed as cases of latent treponemal disease. Because no information is available about the behaviour of the IgM-FTA test in such cases, it was decided to study these patients and also to assess the results of the test in known cases of syphilis in the light of the clinical information available about them.

Received for publication January 16, 1976
Address for reprints: Dr A. E. Wilkinson, V.D.R.L., Ashfield Street, London E.1.

Material and methods

Patients studied

From March to September, 1975, sera from patients attending the Whitechapel Clinic with treated or untreated syphilis or latent treponemal disease were saved and stored at —20°C. The initial diagnosis had been confirmed by specific tests (FTA-ABS, TPHA, or TPI). 337 sera from 278 patients were studied; 169 had syphilis and 109 latent treponemal disease.

Serological tests

VDRL, FTA-ABS, and TPI tests were carried out by the

methods described in PHLS Monograph 1 (1972), and TPHA tests by the method described by Johnston (1972).

IgM-FTA test

Sera were screened at a dilution of 1 in 5 in an ultrasonicate of Reiter treponemes to remove group-reactive antitreponemal antibody (Wilkinson and Wiseman, 1971). Sera giving 1+ or greater fluorescence were titrated out, a 1+ reading being taken as the end-point. Monospecific antihuman IgM conjugates (Hyland, Wellcome Reagents) were used at optimal titres derived from a chessboard titration against known IgM-positive serum. Quantitative tests on dilutions of IgM-positive serum in buffered saline and IgM-negative serum were included in each batch of tests; these control sera were stored in aliquots at —20°C. and used only once. The tests were read on a Leitz Ortholux microscope with an iodine quartz illuminator, a ×54 oil immersion objective, and ×10 oculars. A Balzer FITC 3 interference filter and a 520 barrier filter were used. All the tests were read by the same observer (A. E. W.) without knowledge of the clinical status of the patients. A clinical evaluation was later carried out by P. R.

Rheumatoid factor

Latex tests (Wellco rheumatest, Wellcome Reagents), were carried out at a serum dilution of 1 in 25. Selected positively reacting sera were re-tested by the IgM-FTA test after absorption with an equal volume of insolubilzed human globulin to remove anti-IgG antibody.

Results and discussion

The results of the IgM-FTA tests and their relation to the time since treatment in the various patient groups are shown in Tables I to VI.

IgM-FTA tests were reactive in virtually all the sera from patients with untreated primary, secondary and early latent infections. Early latent refers to patients within the first year of infection. The largest proportion of high titres (1 in 135), was found among patients in the secondary stage (Tables I, II, and III). After treatment of early syphilis, IgM antitreponemal antibody was detected in 24 of 36 specimens examined during the first year after treatment and in four of 26 sera tested more than one year after treatment had been given. These findings agree with those of O'Neill and Nicol (1972).

Müller and Loa (1974) found that twelve of 32 sera from patients with untreated secondary syphilis and seventeen of 43 sera from patients with untreated tertiary syphilis gave negative IgM-FTA tests. When the tests were repeated on the IgM fractions of these sera separated by gel chromatography, all were FTA-IgM positive. These discrepancies may have been due to competition between IgG and IgM, but no information is given about the relative FTA titres of the two immunoglobulins in these sera.

In untreated late latent syphilis the proportion of positive IgM-FTA tests was considerably lower, six out of 26 sera, and the titres also tended to be lower than in untreated early syphilis (Table IV). These patients tended to be older (median age 41 yrs, range 19 to 65) than the smaller group with untreated early latent infections (median age 28 yrs, range 21 to 48). Eleven reactive tests were found in the 44 sera from 37 patients with treated late latent infections. The titres tended to be low and all but

TABLE I *IgM-FTA tests in darkground positive primary syphilis*

			Result						
					Titre				
No. of sera	*Treatment*	*Time since treatment*	*Negative*	*Borderline*	5	15	45	⩾135	*No. reactive*
10	Untreated	—	1	—	2	4	2	1	9/10
20 (17 patients)	Treated	1–11 mths	2	—	3	4	1	—	
		12–23 mths	2		—	—	—	—	
		2–5 yrs	2	1	—	—	—	—	
		>5 yrs	5	—	—	—	—	—	
		Total	11	1	3	4	1	—	8/20

TABLE II *IgM-FTA tests in secondary syphilis*

			Result							
					Titre					
No. of sera	*Treatment*	*Time since treatment*	*Negative*	*Borderline*	5	15	45	⩾135	*Reactive not titred*	*No. reactive*
14	Untreated	—	—	—	1	—	2	9	2	14/14
22 (15 patients)	Treated	1–11 mths	6	1	2	4	1	—	1	
		12–23 mths	1	—	—	—	—	—	—	
		2–5 yrs	2	—	—	—	—	—	—	
		>5 yrs	2	1	—	—	1	—	—	
		Total	11	2	2	4	2	—	1	9/22

TABLE III *IgM-FTA tests in early latent syphilis*

No. of sera	*Treatment*	*Time since treatment*	*Result*						*No. reactive*
			Negative	*Borderline*	*Titre* 5	15	45	⩾135	
8 (7 patients[a])	Untreated	—	—	1	3	2	—	2	7/8
20 (13 patients[b])	Treated	1–11 mths	1	2	4	4	—	—	
		12–23 mths	—	—	—	—	—	—	
		2–5 yrs	3	—	2	—	—	—	
		>5 yrs	3	—	1	—	—	—	
		Total	7	2	7	4	—	—	11/20

[a]7 males, 2 homosexuals [b]10 males, 8 homosexuals

three of the reactive sera came from patients who had been treated within the preceding 2 years. Another marked difference between the groups of patients with early and late latent infections was found in the proportion of males who were homosexuals. Ten of the sixteen males with early latent infections (treated or untreated) were homosexuals, compared with only three of the 35 males with treated or untreated late latent infections.

In the patients classed as having latent treponemal disease, the diagnosis lay between latent syphilis and old yaws. Four (14 per cent.) of the 29 patients with untreated latent treponemal disease gave a history of yaws or had scars which were thought to be due to old yaws. In the treated group of eighty patients the figure was 24 per cent. The IgM-FTA test was positive to a low titre in five sera from the 29 untreated patients and in eleven of the 91 sera from the

TABLE IV *IgM-FTA tests in late latent syphilis*

No. of sera	*Treatment*	*Time since treatment*	*Result*						*No. reactive*
			Negative	*Borderline*	*Titre* 5	15	45	⩾135	
26 (19 patients[a])	Untreated	—	17	3	3	2	1	—	6/26
44 (37 patients[b])	Treated	1–11 mths	11	1	3	2	1	—	
		12–23 mths	6	—	1	1	—	—	
		2–5 yrs	5	—	—	—	—	—	
		>5 yrs	10	—	2	—	1	—	
		Total	32	1	6	3	2	—	11/44

[a]13 males, 2 homosexuals [b]25 males, 5 homosexuals

TABLE V *IgM-FTA test in treated late symptomatic and congenital syphilis*

No. of sera	*Syphilis*	*Time since treatment*	*Result*						*No. reactive*
			Negative	*Borderline*	*Titre* 5	15	45	⩾135	
19 (14 patients)	Late symptomatic	1–11 mths	—	—	—	1	—	—	
		12–23 mths	—	—	1	—	—	—	
		2–5 yrs	3	—	—	1	—	—	
		>5 yrs	8	—	2	3	—	—	
		Total	11	—	3	5	—	—	8/19
26 (23 patients)	Congenital	1–11 mths	1	1	2	—	—	—	
		12–23 mths	—	—	—	1	—	—	
		2–5 yrs	1	—	—	—	—	—	
		>5 yrs	17	1	1	1	—	—	
		Total	19	2	3	2	—	—	5/26

TABLE VI *IgM-FTA tests in latent treponemal disease*

No. of sera	*Treatment*	*Time since treatment*	*Result*						*No. reactive*
			Negative	*Borderline*	*Titre* 5	15	45	⩾135	
37 (29 patients[a])	Untreated	—	27	5	5	—	—	—	5/37
91 (80 patients[b])	Treated	1–11 mths	29	1	4	2	—	—	
		12–23 mths	4	—	—	—	—	—	
		2–5 yrs	10	—	1	—	—	—	
		>5 yrs	33	3	4	—	—	—	
		Total	76	4	9	2	—	—	11/91

[a]4 with history of yaws [b]19 with history of yaws

eighty treated patients. Six of these reactive sera were tested within one year of treatment and 5, 2, or more years after treatment. Two patients with reactive tests had a history of yaws; one (aged 47) was untreated, the second (aged 37) had been treated 3 months previously and a second test after a further 4 months was negative. There were no homosexuals among the males with latent treponemal disease.

No patients with untreated late symptomatic or congenital syphilis attended during the survey. Eight of the nineteen sera from fourteen patients with treated late symptomatic syphilis and five of 26 sera from the 23 adult patients with treated congenital syphilis had reactive IgM-FTA tests. The majority of these were found 2 or more years after treatment; the titres were low, not exceeding 1 in 15.

In studies of the IgM-FTA test in neonatal syphilis, Reimer, Black, Phillips, Logan, Hunter, Pender, and McGrew (1975) have shown that an anti-immunoglobulin G may be produced; this is an IgM antibody which could lead to an erroneous IgM-FTA test. Rheumatoid factor, usually an IgM, has been found in a proportion of adult syphilitic sera. Peltier and Christian (1959) detected RA factor in 11 per cent. of 147 patients; eleven of these had late latent syphilis and three neurosyphilis. Mustakallio, Lassus, and Wager (1967) tested 261 syphilitic sera and found RA factor in 27; these were evenly distributed between treated and untreated cases but were more frequent in the older patients. Lassus (1969) found RA factor in five of 101 patients with primary and nine of 51 with secondary syphilis.

Because of the possibility that RA factor might lead to an apparently positive IgM-FTA test, latex tests were carried out whenever possible on sera which were found to be IgM-FTA positive 2 or more years after treatment. These were done on

TABLE VII *Patients with reactive IgM-FTA tests more than 2 years after treatment*

Patient No.	*Sex*	*Age (yrs)*	*IgM-FTA titre*	*Diagnosis*	*Treated*	*Remarks*
1	M	28	45	Secondary	1968	Promiscuous homosexual. Gc 1970, 1973. VDRL negative since 1970
2	M	34	15	Early latent	1968	Promiscuous homosexual. Gc 1969, 1970 (twice). Contact primary syphilis 1973. NSU 1973. VDRL negative 5 yrs
3	M	32	5, 5	Early latent	1973	Homosexual. Nil to suggest activity. VDRL WR
4	M	63	45	Latent	1963	NSU 1974. Penicillin for chest infection. VDRL 4. Nil to suggest activity
5	M	45	5	Latent	1964	Homosexual. Nil to suggest activity. VDRL 4
6	M	35	5	Latent	1965	Promiscuous homosexual. Nil to suggest activity. VDRL negative
7	M	46	5, 15, 15	Tabes dorsalis	1969	Homosexual. Nil to suggest activity. VDRL 2
8	M	53	5	Glossitis	1953	Nil to suggest activity. VDRL 4
9	M	58	15	Cardiovascular syphilis; tabes dorsalis	1970	Nil to suggest activity. VDRL 4
10	M	45	15	GPI	1967	Nil to suggest activity. VDRL 2
11	M	69	15	Congenital	1961	Nil to suggest activity. VDRL neat
12	F	61	5	Congenital	1968	Nil to suggest activity. VDRL negative
13	M	44	5	Latent treponemal disease	1963[a]	Nil to suggest activity. VDRL neat
14	M	35	5	Latent treponemal disease	1961	Probable re-infection 1974. VDRL neat rising to 32. VDRL now 8
15	F	41	5	Latent treponemal disease	1966	Nil to suggest activity. VDRL neat
16	M	35	5	Latent treponemal disease	1963[a]	Nil to suggest activity. VDRL negative
17	M	28	5	Latent treponemal disease	1971[a]	Nil to suggest activity. VDRL neat

[a]Treated with only a single injection of 2·4 m.u. benzathine penicillin

Patients 1, 2, 3, 5, 7, 9, 10, 11, 12, 15, and 17 (Table VII). Positive results were given by sera from Patients 10, 11, and 17. These sera were absorbed with insolubilized human globulin and the IgM-FTA tests repeated. All three remained positive at the same titre, and the results were therefore presumed to be due to specific antitreponemal IgM antibody and not to an anti-immunoglobulin of IgM class.

IgM antitreponemal antibody seems to disappear within one year after treatment in almost all patients with early or early latent syphilis. O'Neill and Nicol (1972) suggested that this might be an indication of arrest of the disease. In the present series there were seventeen patients in whose sera IgM-FTA antibody was still detectable 2 or more years after treatment (Table VII). Six of these patients were homosexuals (1, 2, 3, 5, 6, and 7); one of these had been a contact of a patient with primary syphilis but there was no clinical evidence of re-infection. Homosexuals are often promiscuous and thus probably liable to exposure to syphilitic infection, but except in this one instance there was no definite evidence that this had occurred. One further patient (No. 14), had other evidence of relapse or re-infection, his VDRL titre having risen from neat to 1 in 32 about a year before the IgM-FTA test was performed. The remaining ten patients showed no clinical evidence to suggest activity of their disease and the persisting positive IgM-FTA tests were unexplained. The number of patients studied is small, but the results with treated late latent or late symptomatic syphilis do not support the view that persistence of IgM antitreponemal antibody is associated with continued activity of the disease as assessed clinically. Conversely, many patients with apparently untreated latent syphilis had negative IgM-FTA tests. In our view these patients should nevertheless be treated.

Although the proportion of sera from patients with untreated latent treponemal disease which were IgM-FTA positive was rather lower than that from those with untreated late latent syphilis, it does not seem that the test assists in distinguishing latent syphilis from old yaws. However, the marked difference in the proportion of positive results in untreated early latent compared with that in late latent syphilis suggests that the test may assist in distinguishing between these two stages. A positive result with a high titre (1 in 45 or above), would favour an early rather than a late latent infection. It might also assist in making a retrospective diagnosis in seropositive patients who have been recently treated for genital lesions without a darkground examination, *e.g.* seamen treated on board ship. A positive test would support a diagnosis of recent early syphilis.

Our thanks are due to the World Health Organization for financial support.

References

ATWOOD, W. G., and MILLER, J. L. (1969) *Arch. Derm.*, **100,** 763

——, —— (1970) *Int. J. Derm.*, **9,** 259

JOHNSTON, N. A. (1972) *Brit. J. vener. Dis.*, **48,** 474

JULIAN, A. J., LOGAN, L. C., and NORINS, L. C. (1969) *J. Immunol.*, **102,** 1250

——, ——, ——, and SCOTTI, A. T. (1971) *Infect. and Immun.*, **3,** 559

LASSUS, A. (1969) *Int. Arch. Allergy*, **36,** 515

LOGAN, L. C., NORINS, L. C., ATWOOD, W. G., and MILLER, J. L. (1969) *J. invest. Derm.*, **53,** 300

MÜLLER, F., and LOA, P. L. (1974) *Infection*, **2,** 127

MUSTAKALLIO, K. K., LASSUS, A., and WAGER, O. (1967) *Int. Arch. Allergy*, **31,** 417

O'NEILL, P., and NICOL, C. S. (1972) *Brit. J. vener. Dis.*, **48,** 460

PELTIER, A., and CHRISTIAN, C. L. (1959) *Arthr. and Rheum.*, **11,** 1

PUBLIC HEALTH LABORATORY SERVICE. Monograph 1, 'Laboratory Diagnosis of Venereal Disease' (1972). H.M.S.O., London

REIMER, C. B., BLACK, C. M., PHILLIPS, D. J., LOGAN, L. C., HUNTER, E. F., PENDER, B. J., and MCGREW, B. E. (1975) *Ann. N.Y. Acad. Sci.*, **254,** 77

WILKINSON, A. E., and WISEMAN, C. C. (1971) *Proc. roy. Soc. Med.*, **64,** 422