Quantitative evaluation of the FTA-ABS-IgM and VDRL test in treated and untreated syphilis

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SUMMARY Observations made on fluorescent treponemal antibody absorption (FTA-ABS) immunoglobulin M (IgM) titres in patients with untreated early syphilis showed non-reactive or weakly reactive results in patients with primary (one of five cases) and secondary (two of 16 cases) lesions. In patients with primary (19.5%) and secondary (15%) syphilis sera remained reactive with increased titres for more than one year after treatment. The respective figures in the results of the Venereal Disease Research Laboratory (VDRL) test were zero in primary and 20% in secondary syphilis. The non-reactive FTA-ABS IgM results may possibly be explained by competitive inhibition of IgM by immunoglobulin G (IgG). The persistence of reactivity in a comparable percentage has been observed by other investigators (Grin *et al.*, 1974; Wilkinson and Rodin, 1976). The current results therefore suggest that FTA-ABS IgM titres are less reliable for assessing the effect of treatment than the course of the VDRL titres. The phenomenon of a decrease in FTA-ABS IgM titres soon after treatment with a later rise before final non-reactivity is a matter for further investigation.

Introduction

The detection of immunoglobulin (IgM) antibodies against *Treponema pallidum* has for some time been considered of value in diagnosing syphilis and assessing the effect of treatment (Bowszyc, 1975; Manikowska-Lesinska *et al.*, 1976; Wilkinson, 1976; Wilkinson and Rodin, 1976; Müller, 1977a, b). Experience suggests that IgM antibodies appear before other immunoglobulins and are generally present in the sera of untreated patients with early syphilis, but can be detected in only 20% of people with untreated late and latent syphilis (Wilkinson and Rodin, 1976). Investigations to confirm this have been made at this institute and have given similar results.

Material and methods

An increasing number of sera (at present more than 100 000 samples a year) are examined at the institute using the automated microhaemagglutination assay

with *T. pallidum* antigen (AMHA-TP) and the Venereal Disease Research Laboratory (VDRL) test. All sera reactive to one of these two tests are further evaluated by the fluorescent treponemal antibody absorption (FTA-ABS) test which is carried out in accordance with international recommendations (United States Public Health Service, 1969).

The microscope used for the test was a Reichert-Zetopan. Fluorescence was induced by a mercury high pressure lamp HBO 200 W, with an excitor filter UG 1 and a barrier filter GG 13 with a Wratten folia 2 B.

The detection of IgM antibodies followed the same principle but a specific monovalent anti-human-IgM labelled with sodium-fluorescein-isothiocyanate was used instead of the polyvalent anti-human globulin. The quantitative FTA-ABS IgM was determined by diluting the samples in a geometrical progression by the factor 3 starting with 1:5, 1:45, etc.

The conjugates were products of Wellcome Laboratories and contained pure sheep anti-human-IgM. A check of the reliability of the reagent with sera of known IgM reactivity was performed before any investigation started.

The VDRL test and the quantitative evaluation of

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N (Persons) = 48 N (Examinations) = 142

Fig. 2 IgM and VDRL in secondary syphilis before and after treatment.

to the FTA-ABS IgM test; the others showed titres between 1: 5 and 1: 3 645 (mean value 1: 282).

One hundred and twenty-six sera were from patients who had received treatment between one month and 34 years before the test. Fifty-three (42.1 %) of these sera were reactive up to dilutions of between 1: 5 and 1: 405. The results of the remaining 73 were non-reactive (62) or weakly reactive (11).

Six of 40 (15 %) samples were reactive more than one year after treatment at dilutions of 1: 5 (1), 1: 15 (2), and 1: 45 (3).

The VDRL test was performed on 16 sera before treatment, all were reactive at titres between 1: 8 and 1: 64 (mean value 1: 34.5). Sixty-two of the samples which had been examined after treatment were non-reactive (47) or weakly reactive (15). Sixty-four sera (51.6 %) were reactive at titres between 1: 1 and 1: 64.

The reactivity persisted for more than one year after treatment in eight of 40 (20 %) specimens, the respective dilutions reached from 1: 1 (3), 1: 2 (2), 1: 4 (2), to 1: 8 (1).

LATENT SYPHILIS

We were unable to distinguish between samples from patients with early latent and late latent syphilis, therefore a precise evaluation cannot be given. In Fig. 3 the FTA-ABS IgM and VDRL titres are compared. The agreement between non-reactive and weakly reactive samples using either method was 61 out of 121 (50.41 %), and 10 further sera (8.26 %) showed good agreement because the outcome of the tests did not deviate from the middle for more than one step. The other 50 results (41.32 %) were scattered (Fig. 3).

Altogether 37 samples (30.6 %) were reactive, 84 were non-reactive (67) or weakly reactive (17).

FOLLOW-UP

Nine patients with secondary syphilis were followed-up. FTA-ABS IgM and VDRL titres showed only partial agreement during the course of observation. The results are shown in Fig. 4. The FTA-ABS IgM titre usually reverted to non-reactivity later than the

VDRL dilutions. The VDRL titre therefore seems to be better for measuring the effect of treatment.

		IgM titre									Total
		-NR	WR	1:5	1:15	1:45	1:135	1:405	1:1215	1:3645	
VDRL titre	-NR	40	10	9	7	1	2				69
	±WR	9	2		2						13
	1:1	6	2	3							11
	1:2	4	1	3				1			9
	1:4	4	2		1			1			8
	1:8	1			2	1					4
	1:16	2		1							3
	1:32	1									1
	1:64			1	1			1			3
		67	17	17	13	2	2	3	-	-	121

Fig. 3 IgM and VDRL titres in latent syphilis.

IGM AND VDRL TITRES SHORTLY AFTER SINGLE DOSE TREATMENT

Case 1

A 25-year-old man presented with a chancre 1.5 cm across in the right inguinal region. Darkfield examination showed *T. pallidum*. A mild Herxheimer reaction began three hours after the injection of 4.8 megaunits benzathine-penicillin with a rise of the axillary temperature to 37.3°C which lasted for

Fig. 4 Succession of IgM and VDRL titres in the sera of nine patients with secondary syphilis.

12 hours, but with no other general or local symptoms. Fig. 5 shows that the IgM titre decreased to zero within three weeks after treatment, but that it later rose to 1:5 and then finally returned to non-reactive or weakly reactive results one month later. The VDRL titre was weakly reactive, apart from 48 hours after the injection of penicillin when it was reactive in neat serum.

Fig. 5 IgM and VDRL titres in the serum of a patient with primary syphilis shortly after penicillin administration.

Case 2

A 77-year-old man presented with a macular rash caused by secondary syphilis. The patient was observed two days before and 11 days after a single injection of 4.8 megaunits of benzathine penicillin (Fig. 6).

A mild Herxheimer reaction began four hours after the injection; the rash became deeper in colour and the axillary temperature rose to 37.7°C where it remained for 12 hours. The FTA-ABS IgM titre increased at first irregularly from 1:135 up to 1:1215 and was then checked at three-hourly intervals after injection of penicillin. The titre decreased steadily until it returned to non-reactivity two days after treatment. However, during the next few days an increase in the FTA-ABS IgM concentration in the serum was observed until the sixth

day when the titre reached a peak of 1:135, after which it dropped to weakly reactive within two days.

The VDRL titre hovered around 1:32 but remained within the margin of error of one dilution until the fifth day after treatment and then declined steadily to 1:8.

Fig. 6 IgM and VDRL titres in the serum of a patient with secondary syphilis shortly after application of 4.8 megaunits of benzathine penicillin.

Both results suggest that the antisyphilitic treatment might influence the FTA-ABS IgM titre by a marked decrease after treatment and a later increase up to a second peak. This phenomenon is a matter for further study but it has proved difficult to find patients who were willing to stay in hospital for long enough to gather data.

Discussion

On long-term observations the FTA-ABS IgM titres showed neither a prompt nor a steady decrease after treatment but remained reactive beyond the first year after treatment at dilutions of 1:5 and more in 19.5% of cases of primary syphilis, and 15.0% of cases of secondary syphilis. Similar results have been reported by others (Grin *et al.*, 1974; Wilkinson, 1976; Wilkinson and Rodin, 1976).

Possible explanations for non-reactive or weakly reactive results in primary and secondary syphilis before treatment could be a competitive inhibition of the specific IgM production or a blocking of the IgM receptors on the surface of the antigen by IgG as explained by Wilkinson (1976) and Müller (1977b).

Contamination of the FTA-ABS IgM reagent with IgG could be a source of error. We are currently trying to establish if this can influence the FTA-ABS IgM results and if so to what extent. The work has not yet finished.

A possible contamination of the conjugate with IgG could have a marked effect on all investigations since IgG induces a more intensive fluorescence than IgM. Nevertheless, the present standard of controls suggests that contamination would only affect the result by one dilution.

The influence of the sorbent on the outcome of the FTA-ABS IgM results has already been stressed (Vozely, 1974; Wilkinson and Johnston, 1975). Müller (1976) demonstrated that only one out of 10 different sorbent preparations produced satisfactory results, all others did not completely absorb non-specific group antigens.

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