

Reactivity in the Venereal Diseases Research Laboratory test and the Mercia[®] IgM enzyme immunoassay after treatment of early syphilis

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Summary: The aim of the study was to compare reactivity in the Mercia immunoglobulin M enzyme immunoassay (IgM EIA) and the Venereal Disease Research Laboratory (VDRL) after treatment of 229 previously untreated patients with early syphilis. At three months, the VDRL and the IgM EIA were negative in 41 (38%) and 71 (62%) cases, respectively; a four-fold or greater decrease in VDRL titre occurred in 106 (99%). At six months, the VDRL and the IgM EIA were negative in 45 (48%) and 69 (71%) patients, respectively; a four-fold or greater decrease in VDRL titre occurred in 88 (95%) and an eight-fold or greater decrease in 80 (86%). At 12 months, the VDRL and the IgM EIA were negative in 35 (70%) and 55 (92%) patients, respectively; a four-fold or greater decrease in VDRL titre occurred in 49 (98%) and an eight-fold or greater decrease in 47 (94%). The Mercia IgM EIA is as sensitive as the VDRL in monitoring treatment of primary syphilis but not as sensitive as the finding of a four-fold or eight-fold decrease in VDRL titre in patients treated for secondary or early latent infection.

Keywords: syphilis, treponemal infection, serodiagnosis

INTRODUCTION

As symptoms and signs of early syphilis regress spontaneously, cure of infection cannot be ascertained by resolution of clinical features. Serological tests for syphilis are therefore required to assess the efficacy of the treatment. Currently, non-treponemal tests such as the Venereal Diseases Research Laboratory (VDRL) or rapid plasma reagin (RPR) tests are used for this purpose. Brown *et al.*¹ showed that after successful treatment the VDRL titre declined four-fold at three months and eight-fold by six months. The data presented by Romanowski *et al.*² indicated that successful treatment for primary and secondary syphilis was in keeping with a four-fold decrease in the RPR test by six months and an eight-fold decrease by 12 months, irrespective of the initial titre. Romanowski *et al.*² also showed that 50% of patients who had been treated for primary syphilis and 22% of those treated for secondary infection showed seroreversion by 12 months.

The VDRL and RPR tests are based on the detection of cardiolipin antibody that is not *Treponema pallidum*-specific, and therefore they may be positive in the absence of active treponemal infection. Such false-positive results are particularly prevalent among HIV-infected patients.³

After successful treatment of syphilis, antitreponemal IgM declines rapidly and uniformly.⁴ Commercial enzyme immunoassays (EIAs) have been developed for the detection of specific antitreponemal immunoglobulin M (IgM). These tests are less labour-intensive and lend themselves more easily to automation than the cardiolipin tests. Their performance, however, has not been compared adequately with that of the non-treponemal tests.

The aim of the present study was to compare the performance of the Mercia[®] IgM EIA and the VDRL test in the assessment of the efficacy of the treatment of early syphilis.

METHODS

Patients

The patient groups and the definitions of primary, secondary and early latent syphilis have been described in detail elsewhere.⁵

Patients were treated with intramuscular benzathine penicillin or, in those with a history of penicillin hypersensitivity, with oral doxycycline in accordance with the published guidelines.⁶ Individuals treated during the study period were invited to attend for repeat serological testing three, six and 12 months after completion of therapy.

Laboratory methods

Heat-inactivated serum was used in performing tests to monitor the efficacy of the treatment. The level of specific

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antitreponemal IgM was detected by the Mercia Syphilis M EIA using undiluted serum. The result of this test is expressed as an index and interpreted according to the manufacturer's instructions; an index of <1 was considered negative and an index ≥ 1 was considered positive.

The VDRL titre was determined by the Murex VDRL carbon antigen assay using undiluted serum and doubling dilutions of serum until an endpoint was obtained: the titre was taken as the last reactive dilution and was expressed numerically, e.g. 1 = positive with undiluted serum only, 2 = positive up to a one in two dilution only, 4 = positive up to a one in four dilution only, etc. Any serum which was non-reactive with undiluted serum was considered negative and scored 0.

In assessing the VDRL response to treatment, the following categories were considered.

- (i) VDRL-negative without a four-fold decrease in titre (denominator is any serum with a pretreatment titre <2).
- (ii) VDRL-negative and a four-fold decrease in titre (denominator is any serum with a pretreatment titre = 2).
- (iii) VDRL-negative and an eight-fold decrease in titre (denominator is any serum with a pretreatment titre >4) but not included in categories (iv) or (v).
- (iv) a four-fold decrease in titre (denominator for this is any serum with a pretreatment titre = 4) but not included in category (iii).
- (v) an eight-fold decrease in titre (denominator for this is any serum with a pretreatment titre >8) but not included in category (iii).

The total VDRL-negative cases were given by adding (i), (ii) and (iii), the total with a four-fold or greater decrease in VDRL titre by adding (ii), (iii), (iv), (v) and the total with an eight-fold or greater decrease by adding (iii) and (v).

The efficacy of the treatment by VDRL titre could not be assessed for those patients with a pretreatment VDRL titre of 2 that remained positive on follow-up.

Statistical methods

The chi-squared test with Yates' modification was used in the analysis of the categorical data entered into a software statistical package (Statgraphics[®]; Manugistics, Rockville, MD, USA).

Table 1 Number of patients studied at intervals after completion of treatment for early syphilis

Stage of syphilis	Number of patients before treatment		Number of patients whose pretreatment VDRL (IgM) test was positive who were followed-up at this interval after treatment		
	VDRL +	IgM +	3 months	6 months	12 months
Primary (n = 89)	60	80	34 (44)	27 (37)	11 (24)*
Secondary (n = 68)	68	68	43 (43)	37 (37)	23 (23) [†]
Early latent (n = 72)	61	53	30 (28)	29 (23)	16 [‡] (13)

*Six patients were HIV infected, 11 were HIV-seronegative and seven patients had not been tested for HIV infection

[†]Seven patients were HIV infected, 15 patients were HIV-seronegative and one man had not been tested for HIV infection

[‡]Three patients were HIV infected, 11 patients were HIV-seronegative and two patients had not been tested for HIV infection

VDRL = Venereal Disease Research Laboratory

RESULTS

Previously untreated patients

Two hundred and twenty-nine previously untreated patients were treated for early syphilis: 222 men and seven women. Two hundred and six patients received benzathine penicillin, and 23 were given doxycycline. Table 1 shows the total number of patients who were VDRL-positive or IgM-positive at the time of treatment as well as those who were successfully followed up after treatment; none had clinical features of persistent infection. As shown in Table 1, the IgM test with 201 (88%) positive at diagnosis had greater potential for monitoring the efficacy of the treatment when all early stages of syphilis are combined than the VDRL test with 189 (83%) positive at diagnosis; this difference is not significant ($\chi^2 = 2.09$; $P > 0.05$). The superior potential of the IgM test in monitoring the treatment efficacy was entirely due to a significant difference in primary syphilis with 80 (90%) positive at diagnosis compared with 60 (67%) for the VDRL ($\chi^2 = 12.08$; $P < 0.0005$). In contrast, the VDRL had greater potential for monitoring therapy in early latent syphilis with 61 (85%) positive at diagnosis compared with 53 (74%) for the IgM test; this difference is not significant ($\chi^2 = 2.06$; $P > 0.05$).

Table 2 shows the proportion of patients who meet the variable criteria for assessing the overall efficacy of the treatment for early syphilis.

At three months post-treatment, the VDRL and the IgM EIA were negative in 41 (38%) and 71 (62%) cases, respectively, a difference that is significant ($\chi^2 = 11.24$; $P = 0.0008$). A four-fold or greater decrease in VDRL titre occurred in 106 (99%) patients, an observation that was more frequent than a negative IgM EIA ($\chi^2 = 90.76$; $P < 0.0001$). At six months post-treatment, the VDRL and the IgM EIA were negative in 45 (48%) and 69 (71%) patients, respectively, a significant difference ($\chi^2 = 9.31$; $P = 0.002$). A four-fold decrease in VDRL titre occurred in 88 (95%) cases, a more frequent observation than a negative IgM EIA ($\chi^2 = 16.55$; $P = 0.0001$). An eight-fold decrease in VDRL titre was also found more often (80 [86%] cases) than a negative IgM assay ($\chi^2 = 5.37$; $P = 0.02$). At 12 months post-treatment, the

Table 2 Number (and percentage) of patients with early syphilis with positive pretreatment Venereal Disease Research Laboratory (VDRL) or IgM meeting the given criteria for cure by three, six and 12 months after treatment

Assessment criteria	Follow-up at:		
	3 months*	6 months [†]	12 months
IgM-negative	71 (62)	69 (71)	55 (82)
VDRL-negative without a four-fold increase	0	1 (1)	1 (2)
VDRL-negative and a four-fold decrease	4 (4)	3 (3)	2 (4)
VDRL-negative and an eight-fold or greater decrease	37 (35)	41 (44)	32 (64)
VDRL-positive with a four-fold decrease	3 (3)	5 (5)	0
VDRL-positive with an eight-fold or greater decrease	62 (58)	39 (42)	15 (30)

*At three months after treatment of primary syphilis, one patient had an increase in VDRL titre from 1 to 4 (IgM EIA negative)

[†]At six months post-treatment, the VDRL titre remained unchanged in three cases of primary syphilis, and in a patient with early latent infection, the titre fell from 2 to 1; in each case the IgM EIA was negative
EIA = enzyme immunoassay

VDRL and the IgM EIA were negative in 35 (70%) and 55 (92%) patients, respectively, a significant difference ($\chi^2 = 7.2$; $P = 0.007$). A four-fold or greater and an eight-fold or greater decrease in VDRL titre was noted in 49 (98%) and 47 (94%) cases, respectively; there is no significant difference between these outcomes and a negative IgM EIA ($\chi^2 = 1.07$ and 0.01 , respectively; $P > 0.05$). Tables 3–5 analyse the different criteria for assessing the efficacy of the treatment for syphilis by stage of disease.

Three months after completion of treatment

Twenty patients (59%) who had been treated for primary syphilis had a negative VDRL; a greater proportion of patients, 37 (84%), had a negative Mercia IgM EIA ($\chi^2 = 4.26$; $P = 0.04$). There was no significant difference in the proportion of individuals whose serum showed a four-fold or greater reduction in VDRL titre (33 [97%]) and those with a negative Mercia IgM EIA ($\chi^2 = 2.12$; $P > 0.05$).

In the case of secondary syphilis, 12 (28%) patients had a negative VDRL test at three months post-treatment, there being no significant difference between this proportion and that of those with a negative Mercia IgM EIA ($\chi^2 = 1.28$; $P > 0.05$). A significantly greater number of patients had a four-fold or greater reduction in VDRL titre than a negative IgM test (43 [100%]) ($\chi^2 = 32.48$; $P < 0.0001$).

There was no significant difference in the proportion of patients with a negative Mercia IgM EIA and a negative VDRL test (16 [57%] and nine [30%], respectively) three months after treatment of early latent infection ($\chi^2 = 3.31$; $P > 0.05$). The proportion of patients whose serum showed at least a four-fold reduction in VDRL titre (30 [100%]), however, was higher than those who had a negative IgM assay ($\chi^2 = 13.70$; $P = 0.0002$).

Six months after completion of treatment

A greater proportion of patients (87%) who had been treated for primary syphilis had a negative Mercia IgM EIA than a negative

Table 4 Number (and percentage) of patients with secondary syphilis with positive pretreatment Venereal Disease Research Laboratory (VDRL) or IgM meeting given criteria for cure by 3, 6 and 12 months after treatment

Assessment criteria	Follow-up at:		
	3 months	6 months	12 months
IgM-negative	18 (42)	19 (51)	19 (83)
VDRL-negative without a four-fold decrease	0	0	0
VDRL-negative and a four-fold decrease	0	0	0
VDRL-negative and an eight-fold or greater decrease	12 (28)	18 (49)	17 (74)
VDRL-positive with a four-fold decrease	0	0	0
VDRL-positive with an eight-fold or greater decrease	31 (72)	19 (51)	6 (26)

VDRL (59%) ($\chi^2 = 4.80$; $P = 0.03$). There was no difference between the proportion of patients who had a four-fold or greater reduction in VDRL titre (23 patients [85%]) and those with a negative Mercia IgM EIA ($\chi^2 = 0.05$; $P > 0.05$). Similar proportions of patients had an eight-fold or greater reduction in VDRL titre (19 [70%]) and a negative IgM test ($\chi^2 = 1.61$; $P > 0.05$).

Similar proportions of patients treated for secondary syphilis had a negative Mercia IgM EIA (51%) and a negative VDRL test (49%). A greater proportion of these patients had at least a four-fold (and eight-fold) reduction in VDRL titre (37 [100%]) in each case than a negative IgM assay.

A higher proportion of patients treated for early latent syphilis had a negative Mercia IgM EIA (78%) than a negative VDRL test (11 [38%]) ($\chi^2 = 6.90$; $P = 0.008$). The proportion of patients who had at least a four-fold or an eight-fold decrease in VDRL titre was similar to those who had a negative IgM test (28 [97%] and 24 [83%], respectively) ($\chi^2 = 2.60$ [$P > 0.05$] and $\chi^2 = 0.003$ [$P > 0.05$], respectively).

Twelve months after completion of treatment

In all cases of primary syphilis, both the Mercia IgM EIA and the VDRL test were negative, and 10 of the 11 patients showed a four-fold or an eight-fold decrease in VDRL titre.

Table 3 Number (and percentage) of patients with primary syphilis with positive pretreatment Venereal Disease Research Laboratory (VDRL) or IgM meeting given criteria for cure by 3, 6 and 12 months after treatment

Assessment criteria	Follow-up at:		
	3 months*	6 months [†]	12 months
IgM negative	37 (84)	32 (87)	24 (100)
VDRL-negative without a four-fold decrease	0	1 (4)	1 (9)
VDRL-negative and a four-fold decrease	4 (12)	3 (11)	2 (18)
VDRL-negative and an eight-fold or greater decrease	16 (47)	12 (44)	8 (73)
VDRL-positive with a four-fold decrease	3 (9)	1 (4)	0
VDRL-positive with an eight-fold or greater decrease	10 (29)	7 (26)	0

*At three months post-treatment, one patient had an increase in VDRL titre from 1 to 4 (IgM EIA-negative)

[†]At six months post-treatment, the VDRL titre remained unchanged in three cases; in each case the IgM EIA was negative
EIA = enzyme immunoassay

Table 5 Number (and percentage) of patients with early latent syphilis with positive pre-treatment Venereal Disease Research Laboratory (VDRL) or IgM meeting given criteria for cure by 3, 6 and 12 months after treatment

Assessment criteria	Follow-up at:		
	3 months	6 months*	12 months
IgM-negative	16 (57)	18 (78)	12 (92)
VDRL-negative without a four-fold increase	0	0	0
VDRL-negative and a four-fold decrease	0	0	0
VDRL-negative and an eight-fold or greater decrease	9 (30)	11 (38)	7 (44)
VDRL-positive with a four-fold decrease	0	4 (14)	0
VDRL-positive with an eight-fold or greater decrease	21 (70)	13 (45)	9 (56)

*At six months post-treatment, the VDRL titre fell from 2 to 1; the Mercia IgM EIA was negative
EIA = enzyme immunoassay

There was no difference in the proportion of patients with a negative VDRL (74%) and those with a negative IgM EIA (83%) treated for secondary syphilis ($\chi^2 = 0.13$; $P > 0.05$). A four-fold and an eight-fold or greater decreases in VDRL titre were found in all 23 patients. One man who was HIV-infected had a persistently positive VDRL with only a one tube difference from the pretreatment titre (titre 16; initial titre 32); the Mercia IgM EIA was negative. There was no history suggesting re-infection and there were no clinical findings suggestive of active infection.

Patients who had been treated for early latent syphilis were more likely to have a negative Mercia IgM EIA (92%) than a negative VDRL test (44% of 16) ($\chi^2 = 5.49$; $P = 0.02$). All 16 patients had an eight-fold or greater reduction in VDRL titre.

Re-infected patients

This group consisted of 25 patients (all male) who became re-infected during the study period. Patients were identified as having been re-infected at a median (range; interquartile range) interval of 1.25 years (0.25–26 years; 4.0 years) from the previous infection.

The screening Murex immune capture enzyme immunoassay (ICE EIA) and the *T. pallidum* particle agglutination test were positive in all cases. Both the VDRL test and the IgM EIA were positive in 18 men; the VDRL was positive and the IgM EIA was negative in six men, and in one case, the VDRL test was negative, but the IgM EIA was positive. Seroconversion from a negative VDRL was noted in 15 patients in whom the results of serology were available after treatment of the initial infection.

DISCUSSION

Cardiolipin antibody tests are used for the assessment of the efficacy of the treatment of early syphilis. Brown *et al.*¹ showed that after successful treatment the VDRL titre declined four-fold at three months and eight-fold by six months. The current findings are in keeping with these observations. The data presented by Romanowski *et al.*² indicated that successful treatment for primary and secondary syphilis was in keeping with a four-fold decrease in the RPR test by six months and an eight-fold decrease by 12 months, irrespective of the initial titre. Again the current data confirm this finding.

There are some differences between the VDRL results presented here and those presented by Romanowski *et al.*² with respect to the RPR test. One hundred percent, 74% and 44% of the current patients treated for primary, secondary and early latent syphilis, respectively, had a negative VDRL test 12 months after completion of therapy, compared with their finding of 50%, 22% and 13% negativity, respectively, in the RPR test. The observed difference may reflect differences in the pretreatment titres between the two studies. Romanowski *et al.*² showed that the seroreversion rates depend on the initial RPR titre, the higher the pretreatment titre, the lower the probability of seroreversion by 12 months. This may explain the findings with respect to primary syphilis: the initial VDRL titres in the present cohort⁴ were lower than those in the latter study (25% versus 50% having pretreatment titres of between 16 and 128). This factor, however, cannot account for the difference in seroreversion rates with respect to secondary and early latent syphilis when the difference in the proportion of patients having pretreatment titres of between 16 and 128 was not significant (88% in this series

and 75% in patients with secondary syphilis, and 48% and 50%, respectively, in those with early latent infection).

IgM reactivity has been previously shown to decline rapidly after the treatment of early syphilis,⁴ and this was also found in the present study, particularly with respect to primary infection. The Mercia IgM EIA became negative sooner than the VDRL after treatment of primary syphilis, but by 12 months both tests were negative in all patients. This test therefore appears to be valuable in assessing the efficacy of the treatment of primary infection, particularly when the pretreatment VDRL is negative. Although the IgM assay became negative sooner than the VDRL test in patients treated for early latent infection, 8% of patients had a positive result 12 months post-treatment. This may be explained by treatment being given at a later stage of infection than for primary syphilis (the duration of infection in patients with latent infection is often impossible to determine), a hypothesis that is supported by the finding that there was no difference in the proportion of patients with a negative VDRL and IgM assay at each post-treatment interval in those with secondary infection. In assessing treatment efficacy, the Mercia IgM EIA was less sensitive than the finding of a four-fold or an eight-fold decline in VDRL titre in patients 12 months after treatment for secondary and early latent infection.

Four patients had pretreatment VDRL titres of 2 or 1; seroreversion 6–12 months after treatment did not occur, and in one man, the titre increased from 1 to 4. In each case, the Mercia IgM EIA was negative, having been positive before treatment. This finding suggests a place for the IgM test in assessing the treatment efficacy in sero-fast patients.

Re-infection was associated with a positive VDRL in 96% of cases although we do not know how many showed a four-fold increase in titre, which is indicative of re-infection. Nevertheless, all 15 patients in whom the VDRL result after treatment of the initial infection was known seroconverted. This suggests that the VDRL is more sensitive in detecting re-infection than the IgM EIA, which was positive in 76% of cases. Others have also found an IgM EIA assay to have low sensitivity (53%) in detecting re-infection.⁷

In this series of cases, HIV infection did not appear to influence the serological patterns. Rolfs *et al.*⁸ noted that HIV-infected patients were more likely to have serological treatment failure at six months than non-HIV-infected patients. They also found that the RPR titre decreased more slowly in the former group of patients. In a large series of HIV-infected patients with syphilis, Ghanem *et al.*⁹ found that infected individuals were at a higher risk of serological failure than those who were not HIV-infected. Although this was not the finding in the present study, only a small number of HIV-infected individuals were studied. A persistently positive VDRL test was noted in one man in the current series and the Mercia IgM EIA was negative. Re-infection or treatment failure was not considered to be an explanation. The result may reflect the polyclonal B-cell activation found in HIV infection.¹⁰ If this finding is confirmed in larger series of HIV-infected individuals, the Mercia IgM EIA test may prove to be valuable in assessing the efficacy of the treatment of early syphilis.

In conclusion, the Mercia IgM EIA appears to be as sensitive as the VDRL test in ascertaining the efficacy of the treatment of primary syphilis, but not as sensitive as the finding of a four-fold or eight-fold decrease in VDRL titre in patients treated for secondary or early latent infection. The test is less useful in detecting re-infection. It is important to note that the findings reported in this study may not be applicable to other IgM-specific EIAs.

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REFERENCES

- 1 Brown ST, Zaidi A, Larsen SA, Reynolds GH. Serological response to syphilis treatment. A new analysis of old data. *JAMA* 1985;**253**:1296-9
- 2 Romanowski B, Sutherland R, Fick GH, Mooney D, Love EJ. Serologic response to treatment of infectious syphilis. *Ann Int Med* 1991;**114**:1005-9
- 3 Geusau A, Kittler H, Hein U, Dangl-Erlach E, Stingl G, Tschachler E. Biological false-positive tests comprise a high proportion of Venereal Disease Research Laboratory reactions in an analysis of 300,000 sera. *Int J STD AIDS* 2005;**16**:722-6
- 4 Baker-Zander SA, Roddy RE, Handsfield HH, Lukehart SA. IgG and IgM antibody reactivity to antigens of *Treponema pallidum* after treatment of syphilis. *Sex Transm Dis* 1986;**13**:214-20
- 5 McMillan A, Young H. Qualitative and quantitative aspects of the serological diagnosis of early syphilis. *Int J STD AIDS* 2008;**19**:620-4
- 6 UK National Guidelines on the management of syphilis 2007 Draft 3. *Clinical Effectiveness Group, British Association for Sexual Health and HIV*. www.bashh.org/guidelines/draft/SyphilisGuideline2007Draft.pdf
- 7 Lefevre JC, Bertrand MA, Bauriaud R. Evaluation of the Captia enzyme immunoassays for detection of immunoglobulins G and M to *Treponema pallidum* in syphilis. *J Clin Microbiol* 1990;**28**:1704-7
- 8 Rolfs RT, Joesoef MR, Hendershot EF, et al. A randomized trial of enhanced therapy for early syphilis in patients with and without human immunodeficiency virus infection. *N Engl J Med* 1997;**337**:307-14
- 9 Ghanem KG, Erbeling EJ, Wiener ZS, Rompalo AM. Serological response to syphilis treatment in HIV-positive and HIV-negative patients attending sexually transmitted diseases clinics. *Sex Transm Infect* 2007;**83**:97-101
- 10 Jacobson DL, McCutchan JA, Spechko PL, et al. The evolution of lymphadenopathy and hypergammaglobulinemia are evidence for early and sustained polyclonal B lymphocyte activation during human immunodeficiency virus infection. *J Infect Dis* 1991;**163**:240-6

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