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Treponema pallidum immune adherence test for serodiagnosis of syphilis

3: Clinical significance and evaluation of treatment

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SUMMARY Antibody titres were measured in patients with clinical syphilis, and the effect of treatment on the results of the *Treponema pallidum* immune adherence (TPIA) test is reported. In the *Treponema pallidum* haemagglutination (TPHA) test little change in antibody titre occurred after treatment while in the fluorescent treponemal antibody-absorbed (FTA–ABS) test only a slight decrease occurred. The decrease in the antibody titre in the TPIA test was similar to that in the glass plate test, but the findings were different. From the results of gel filtration of sera obtained from patients with syphilis at different intervals after treatment it was apparent that the decrease in antibody titre after treatment mainly concerned the IgM antibody; thus, because of the high sensitivity of the TPIA test to IgM antibody the test is useful in evaluating the effect of treatment.

Introduction

In many countries the diagnosis of clinical syphilis is based on the results of serological tests. Various sensitive techniques have been described using cardiolipin or *Treponema pallidum* as antigen. One problem still remaining is that of monitoring the effect of treatment. The lipoidal antigen tests have mainly been used for this, but they have the disadvantage of giving biological false-positive (BFP) reactions, which arise when cardiolipin is used as the antigen. A serological test is, therefore, needed which has a high specificity—and thus no BFP problem—and which is also technically simple to use.

Because the *T. pallidum* haemagglutination (TPHA) test mainly detects IgG antibody this method cannot distinguish between antibodies produced in treated and untreated cases of syphilis. Another problem is its lack of sensitivity at the beginning of the infection (Lesiński *et al*, 1974; Robertson *et al*, 1975). It has also been reported that the TPHA test detects the antibodies produced in yaws and that false-positive reactions occur in leprosy (LeClair, 1971; Garner *et al*, 1972 and 1973).

In contrast, the fluorescent treponemal antibody-absorbed (FTA–ABS) test reported by Hunter *et al* (1964) detects early antibody, and many workers regard it as a final diagnostic technique. It has, however, the disadvantage that training is required for reading the fluorescent intensity, and false-positive reactions have been reported for this test (Kraus *et al*, 1970a, b). It has also been confirmed that the FTA–ABS–Ig test generally used for diagnosis has a high degree of correlation with the TPHA test (Lesiński *et al*, 1974).

Of the serological tests using *T. pallidum* as antigen there have been many reports on the high specificity of the *T. pallidum* immobilisation (TPI) test (Carpenter *et al*, 1956; Lesiński *et al*, 1974). Since the evaluation of the test is made on the basis of the motility of the organisms, however, the technique is too difficult and expensive for routine use.

In our previous report (Tanaka *et al*, 1978b) the *T. pallidum* immune adherence (TPIA) test, as a diagnostic technique for syphilis, was characterised by a high sensitivity to IgM antibody. In this study patients with clinical syphilis were followed up from the beginning of treatment to determine its effect on TPIA antibody.

Material and methods

SYPHILITIC SERA

The sera were obtained from the dermatology department of the Tokyo Metropolitan Okubo Hospital. All sera were inactivated at 56°C for 30 minutes before use.

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SEROLOGICAL TESTS

The TPIA test was performed as previously described (Tanaka *et al*, 1978a). The glass plate test was carried out by means of a modification of the Venereal Disease Research Laboratory (VDRL) test (Mizuoka *et al*, 1973). The FTA–ABS test reagent was supplied by Fujizoki KK, Tokyo.

GEL FILTRATION

Gel filtration was performed on Sephadex G-200 column (1·0 × 45 cm) using 0·8 ml of patients' sera. Veronal buffer (0.07 mol/l; pH 7·3) containing 0·15 mol/l NaCl was used as eluent.

Results

RESPONSE OF ANTIBODY TITRES AFTER TREATMENT

Figures 1 and 2 show changes in the antibody titre after treatment in patients with clinical syphilis. In Figure 1 the antibody titre of a patient with primary syphilis treated with 1·2 g acetyl-spiramycin daily for 364 days is shown. Antibody tests were performed for 670 days but no changes in the TPHA titre were seen. Antibodies detected by the glass plate and TPIA tests increased temporarily just after treatment but rapidly decreased thereafter. From day 51 the glass plate test gave negative antibody results. The TPIA test, however, still gave positive antibody results at a serum dilution of 1/2 on day 670. In the FTA–ABS test the antibody titre fell more slowly than in the other two tests.

Figure 2 shows the findings in two patients with secondary syphilis. Both patients were given bicillin 3·6 g daily. For Case 1 treatment was stopped on day 441 and for Case 2 on day 204. The TPHA titre decreased slightly for Case 2 but did not change for Case 1. In the glass plate test the antibody titre of Case 1 decreased and the results became negative on day 148. For Case 2, however, a low titre was detected on day 200 after the start of treatment, and the results remained positive. A rapid decrease in TPIA titre was observed for both patients as treatment progressed, but the results remained positive in both cases. In the FTA–ABS test the titre decreased for a short while, but thereafter a constant value was obtained. After the completion of treatment the antibody titre of Case 2 increased temporarily in three of the tests (excluding the TPHA test), but no clinical symptoms were found during the period of testing. The antibody titres then decreased again without treatment. The reason for this is not known, but the patient denied the possibility of reinfection. The results for the other patients are shown in Figure 3.

In conclusion, in the cases of clinical syphilis described, the TPHA titre showed little change after treatment, but both the TPIA titre and the glass plate titre decreased. The FTA–ABS titre decreased gradually after treatment, but the rate of decrease was considerably slower than in the TPIA test. Differences in the reactivities of the TPIA and TPHA tests were investigated by gel filtration.

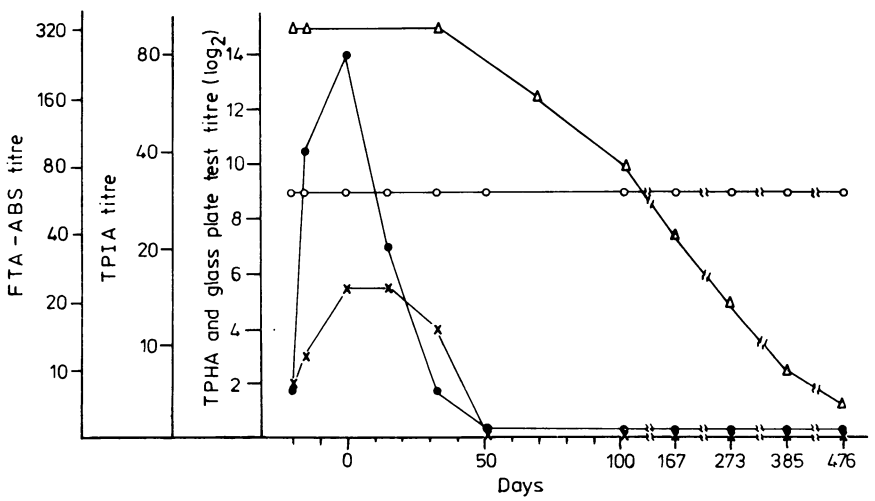

Fig. 1 *Antibody titre after treatment in patient with primary syphilis*
●——● in TPIA test; o——o in TPHA test; x——x in glass plate test; △——△ in FTA–ABS test

ANTIBODY DISTRIBUTION AFTER TREATMENT

Patients' sera obtained at varying intervals after treatment were subjected to gel filtration on Sephadex-200, and antibody titres in the fractions were estimated by means of the glass plate, TPHA, and TPIA tests. The results of sera from a patient with primary syphilis before treatment and on days 33, 101, 168, and 273 after starting treatment are shown in Figure 4. The TPIA results were expressed as IA indices for each of the fractions (Tanaka *et al*, 1978a). The TPIA activity before treatment was distributed in the 19S and 7S fractions but the activity was particularly high in the 19S fraction. As treatment progressed the TPIA activity shifted to the 7S fraction, and on day 101 there was still activity in the 19S fraction but thereafter only in the 7S fraction. The TPHA activity before treatment was distributed in both fractions but mainly in the 7S fraction. After treatment was started the TPHA activity shifted to the 7S fraction, and on day 101 it was almost solely in the 7S fraction. In the glass plate test antibody titre in each fraction was low due to dilution; some activity was detected in the 19S fraction before treatment but on day 33 after treatment the activity had shifted to the 7S fraction.

The results for two patients with secondary syphilis are shown in Figures 5 and 6. In Case 1 TPIA and TPHA reactivity were present in the 19S and 7S fractions, but mainly in the 19S fraction, on day 7 after treatment. The glass plate antibody was found only in the 7S fraction (Figure 5). Figure 6 shows the results for Case 2 before treatment and on days 13 and 61 after treatment. This patient had secondary syphilis as did Case 1, but the TPHA activity was almost all in the 7S fraction, while the TPIA antibody and glass plate antibody were also detected in the 19S fraction. As in the patients with primary syphilis both these cases showed shifts of the TPIA and glass plate activity to the 7S fraction after treatment, and in Cases 1

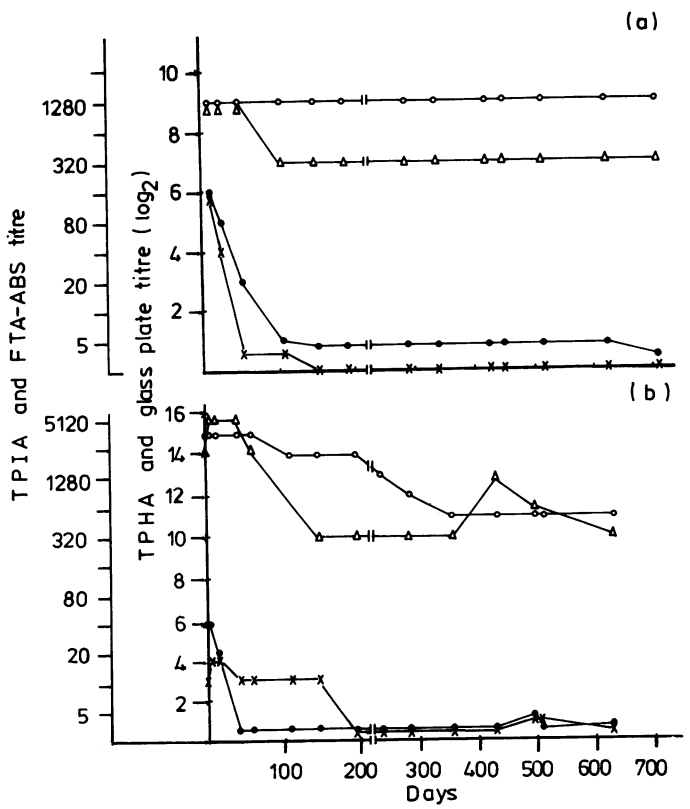

Fig. 2 *Antibody titre after treatment in two patients with secondary syphilis* (*a*) *Case* 1 (*b*) *Case* 2
●——● in TPIA test; o——o in TPHA test; x——x in glass plate test; △——△ in FTA–ABS test

and 2 on days 146 and 61 respectively the TPIA activity was detected only in the 7S fraction.

Discussion

In our previous report we considered that the quantitative TPIA test gave a better estimation of changes in the antibody titre after treatment (Tanaka *et al*, 1978b). This view was substantiated in these experiments. Of the two tests using treponemal antigen the antibody titre in the TPHA test showed almost no change after treatment but that in the TPIA test showed a decrease.

O'Neill *et al* (1972) reported that IgM antibody was detected in all untreated cases of early syphilis but only 12% of the treated cases gave positive results with the FTA–ABS–IgM test, and the IgM antibody decreased after treatment. Oxelius *et al* (1969), Johnston (1972), and Wilkinson and Rodin (1976) also considered that the FTA–ABS–IgM test was suitable for evaluating treatment results. In our experiments similar results were obtained by gel filtration of patients' sera at varying intervals after treatment; the antibody distribution was located mainly in the 19S fraction when the patients were untreated, but as treatment progressed the activity in the 19S fraction decreased and gradually shifted to the 7S fraction. This phenomenon was especially noteworthy in the TPIA test and thus shows that this method is useful in evaluating the results of treatment.

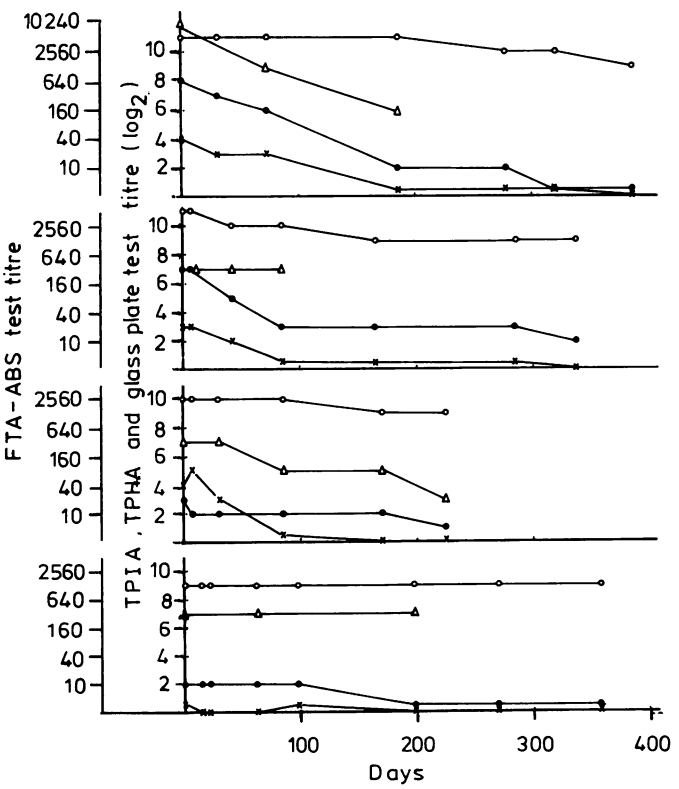

Fig. 3 *Antibody titre after treatment in four patients with secondary syphilis*
●——● in TPIA test; o——o in TPHA test; x——x in glass plate test; △——△ in FTA–ABS test

The decrease in TPIA titre was very similar to that in the glass plate test except that antibodies still remained in the TPIA test after results to the glass plate test had become negative (Figure 1). This difference may have been because the TPIA test was more sensitive for detecting IgG antibody.

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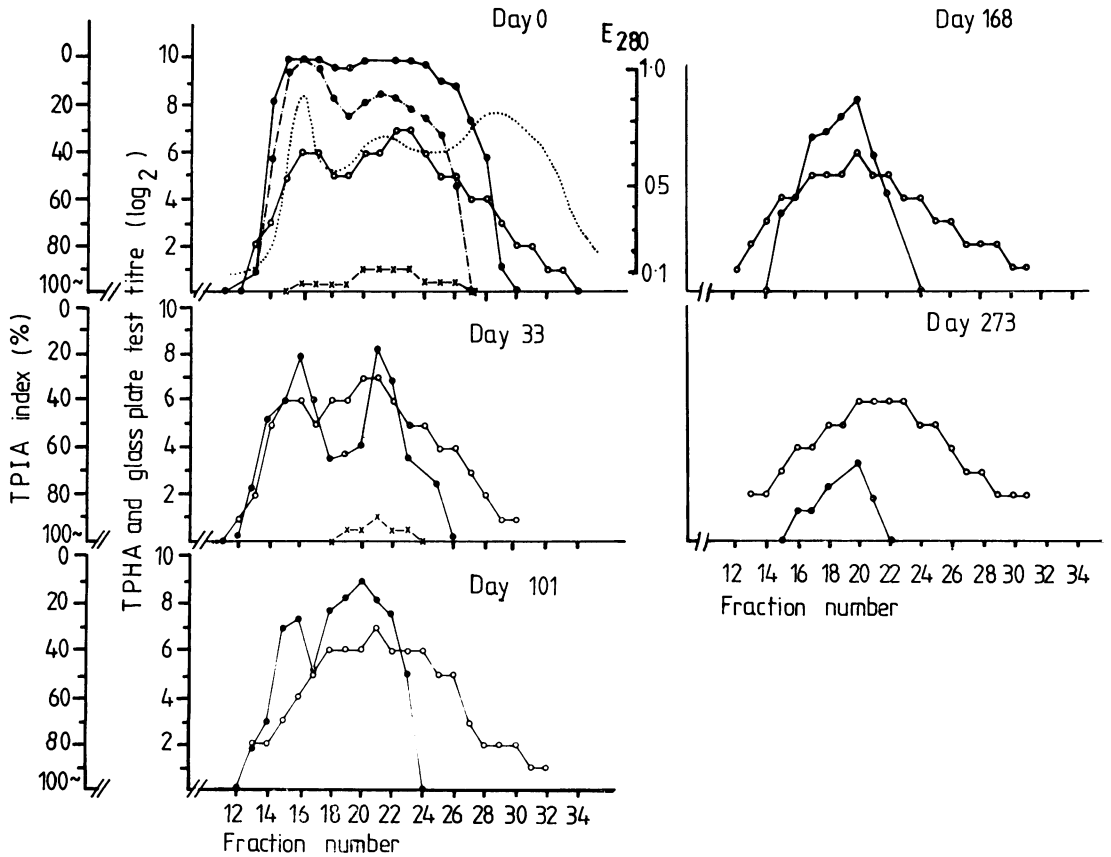

Fig. 4 *Antibody distribution after treatment in patient with primary syphilis*
●——● in TPIA test; o——o in TPHA test; x——x in glass plate test; △——△ in FTA–ABS test; ●—·—·—● in TPIA test with 1/2 serum dilution; ....OD at 280 nm

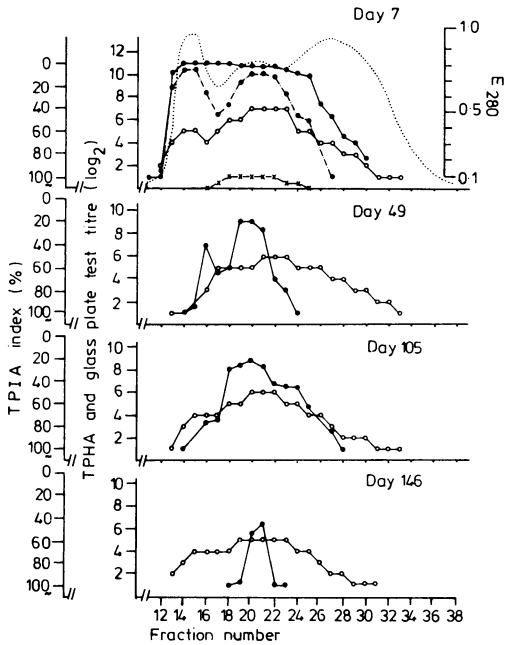

Fig. 5 *Antibody distribution after treatment in patient with secondary syphilis* (*Case* 1)
●——● in TPIA test; o——o in TPHA test; x——x in glass plate test; △——△ in FTA–ABS test; ●—·—·—● in TPIA test with 1/2 serum dilution; ....OD at 280 nm

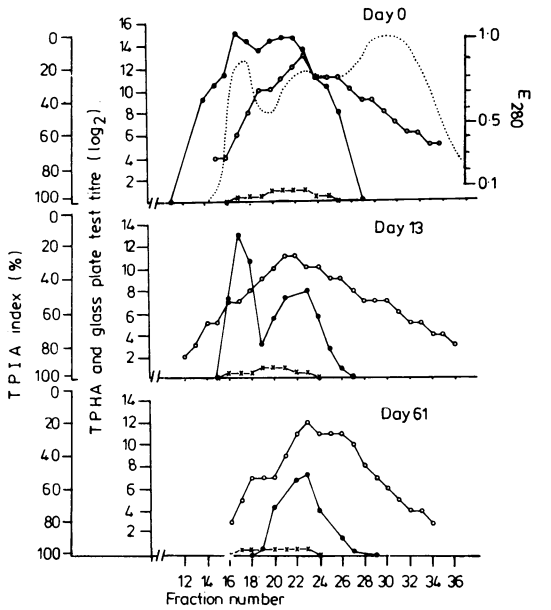

Fig. 6 *Antibody distribution after treatment in patient with secondary syphilis* (*Case* 2)
●——● in TPIA test; o——o in TPHA test; x——x in glass plate test; △——△ in FTA–ABS test; ....OD at 280 nm

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