Treponema pallidum immune adherence test for serodiagnosis of syphilis

2: Comparison with glass plate, TPHA, and FTA-ABS tests

SHINOBU TANAKA,* TAKAKAZU SUZUKI,* TAKEJI NUMATA,* AND KAZUNORI TSUYUKI†

From the *Department of Immunology, School of Hygienic Sciences, Kitasato University, and the †Department of Microbiology, Public Health Institute of Yokohama City, Japan

SUMMARY Using sera from 340 patients with syphilis the *Treponema pallidum* immune adherence (TPIA) test was compared with the glass plate, *T. pallidum* haemagglutination (TPHA), and fluorescent treponemal antibody-absorbed (FTA-ABS) tests. The results of the TPIA test agreed with those of the glass plate, TPHA, and FTA-ABS tests in 65%, 82%, and 73% of cases respectively. In the quantitative TPIA test no significant correlation with the other tests was observed, and it is, therefore, concluded that the TPIA test has highly individual characteristics. From gel filtration the particular feature of the test was its high sensitivity to the IgM antibody. The TPIA test thus appears to be suitable for estimating antibody in the early stages of the disease.

Introduction

No work has been done since Olansky *et al* (1954) investigated the clinical significance of the *Treponema pallidum* immune adherence (TPIA) test. The reasons for this are that the techniques of the test have not yet been fully established and that there has been a notable development in the use of other tests, such as the fluorescent treponemal antibody-absorbed (FTA-ABS) and *Treponema pallidum* haemagglutination (TPHA) tests, which use *T. pallidum* antigen.

In our previous report (Tanaka *et al*, 1978) we described an improved method for the TPIA test as originally reported by Nelson (1953) and evaluated it in the diagnosis of syphilis. This paper gives the results of a further evaluation of the TPIA test compared with the glass plate (modified Venereal Research Laboratory test), TPHA, and FTA-ABS tests.

Material and methods

SYPHILITIC SERA

The sera were obtained from the dermatological department of the Tokyo Metropolitan Okubo

Address for reprints: Dr S. Tanaka, Division of Inflammatology, Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113, Japan

Received for publication 29 March 1978

Hospital and the urological department of the Metropolitan Taito Hospital. All sera were inactivated at 56°C for 30 minutes before use.

TPIA TEST

The technique reported previously was used (Tanaka *et al*, 1978), the results being expressed as IA indices. Indices of 25% or less were positive, those of 50% or more were negative, and those in between were doubtful-positive (qualitative test). The results of the quantitative test were expressed as the maximum dilution which gave IA indices of 25% or less.

SEROLOGICAL TESTS

The TPHA (microtitre method), glass plate (Mizuoka *et al*, 1973), and FTA-ABS tests were used.

GEL FILTRATION

Two types of Sephadex G-200 column (2.4 × 100 and 1.0 × 45 cm) were used. Veronal buffer (0.07 mol/l; pH 7.3) containing 0.1 mol/l NaCl was used as eluent.

Results

COMPARISON OF TPIA TEST WITH OTHER SEROLOGICAL TESTS

The sera from 340 patients with syphilis were tested by the glass plate, TPHA, and TPIA tests.

Sera from 148 of these patients were also examined by the FTA-ABS test. The results of the TPIA test agreed with the results of the glass plate, TPHA, and FTA-ABS tests in 65%, 81.2%, and 73% of cases respectively (Table 1). It is noteworthy that 15.9% of the sera giving positive results to the TPIA test gave negative results to the glass plate test.

Table 1 Correlation of the results of the TPIA test with those of the glass plate, TPHA, and FTA-ABS tests

Tests	TPIA test					
	Positive		Doubtful-positive		Negative	
	No.	%	No.	%	No.	%
Glass plate						
Positive	210	61.8	28	8.2	23	6.8
Negative	54	15.9	14	4.1	11	3.2
TPHA						
Positive	258	75.9	31	9.1	16	4.7
Negative	6	1.8	11	3.2	18	5.3
FTA-ABS						
Positive	104	70.3	27	18.2	9	6.1
Negative	2	1.4	2	1.4	4	2.7

Figures 1 and 2 show the results of the qualitative and quantitative TPIA tests respectively compared with those of the other serological tests. Compared with the TPHA test, which showed the highest correlation with the qualitative TPIA test, titres in the quantitative TPIA test showed a wide range even in the groups of sera with the same TPHA titres; thus it was assumed that the correlation was low (Figure 2). The same trends were also observed between the results of the TPIA test and those of the glass plate and FTA-ABS tests. Many sera which gave strongly positive results to the qualitative TPIA test showed low titres in the glass plate test, and many sera which gave negative results to the glass plate test had high titres in the quantitative TPIA test (Figures 1 and 2). The differences in the reactivity of the two tests were even more marked. In the quantitative TPIA test the correlation with the FTA-ABS test was low although treponemal antigen was used in both tests. This is because many results in the doubtful-positive (25–50%) range of the TPIA test were clearly positive in the FTA-ABS test (Table 1).

Fig. 1 Relationship between qualitative TPIA test and other tests

Table 2 shows the results of various tests on sera from untreated patients. Cases 1–8 were diagnosed to be in the primary stage of syphilis and had some clinical symptoms; Cases 9–19 were in the secondary stage; Cases 20–22 had no obvious symptoms; and Cases 23–25 had latent syphilis. The sera from Cases 1 and 5 gave positive results to only the TPIA test and the TPIA and FTA–ABS tests respectively. In Case 22 serum antibodies were initially absent, but after 10 days gave positive results to the glass plate and TPIA tests. Since sera from Cases 20 and 21 initially gave positive results to only the glass plate test these results were suspected of being biological false-positive results. Later, sera from these two cases gave a doubtful-positive result and a positive result respectively in the TPIA test.

GEL FILTRATION OF SYPHILITIC SERUM

From the results of the comparisons of the various serological tests it is clear that the TPIA test has its own particular characteristics. The TPIA, TPHA, glass plate, and FTA–ABS tests were carried out on

the fractions obtained by gel filtration on Sephadex G-200, and the distribution of antibody activity in the 7S and 19S fractions was investigated. Figure 3 shows the results for patients with syphilis. The TPHA antibody activity presented mainly in the 7S fraction, while the antibody activities in the other tests were distributed in the 7S and 19S fractions. When serum treated with 2 mercapto-ethanol (Miller and Metzger, 1965) was subjected to gel filtration almost no change in TPHA activity was observed, but the antibody in the 19S fraction had disappeared in the TPIA test (Figure 4). As the titre of this serum was low in the glass plate test the distribution of antibody in the test could not be determined. When other sera which had higher titres were tested similar results were obtained in the glass plate test. It was, therefore, concluded that in the TPIA and glass plate tests reactivity was predominantly with IgM antibody.

Discussion

In the previous paper an improved method of the TPIA test was described (Tanaka *et al*, 1978).

Fig. 2 Relationship between quantitative TPIA test and other tests

Fig. 3 Distribution of antibody activity in fractions by gel filtration on Sephadex G-200 in patients with syphilis
x—x in glass plate test; o—o in TPHA test; ●—● in TPIA test; △—△ in FTA-ABS-IgG test;
▲.....▲ in FTA-ABS-IgM test;OD at 280 nm

Fig. 4 Effect of 2-mercaptoethanol treatment for antibody activity in fractions by gel filtration on Sephadex G-200
(a) untreated (b) treated ●—● in TPIA test; o—o in TPHA test; OD at 280 nm

Table 2 Serum antibody titres in patients with untreated syphilis

Case no.	Tests					Diagnosis of syphilis
	Glass plate	TPHA (log ₂)	TPIA		FTA-ABS	
			Index (%)	Titre		
1	0	6	2.9	20	NT	Primary
2	0	10	24.5	1	320	Primary
3	4	9	0	5	320	Primary
4	4	7	0	20	160	Primary
5	0	3	26.8	±	10	Primary
6	4	6	0	32	40	Primary
7	64	10	0	128	NT	Primary
8	64	6	0	128	NT	Primary
9	64	9	0	160	1280	Secondary
10	8	15	0	40	2560	Secondary
11	16	10	0	5	320	Secondary
12	16	11	0	256	10 240	Secondary
13	1	9	0	4	320	Secondary
14	8	11	0	128	320	Secondary
15	32	10	0	128	1280	Secondary
16	32	11	0	256	NT	Secondary
17	1	10	0	2	NT	Secondary
18	64	10	0	512	NT	Secondary
19	128	12	0	128	NT	Secondary
20	1	2	> 100	0	NT	
	2	6	32.4	±	5	*
21	2	6	30.5	±	NT	
	8	6	10.6	1	20	†
22	0	5	> 100	0	NT	
	2	5	7.0	1	NT	‡
23	4	9	7.5	5	NT	Latent
24	8	12	0	20	NT	Latent
25	2	9	3.1	5	NT	Latent

NT not tested

*Positive results nine days later

†Positive results 14 days later

‡Positive results 10 days later

In this study, the correlation between the TPIA test and the glass plate, TPHA, and FTA-ABS tests was investigated. Firstly, in a comparison of the TPIA and glass plate tests the agreement of results was found to be low, and no marked correlation was observed. This is reasonable when it is considered that the glass plate test detects anti-cardiolipin antibody and the TPIA test detects anti-treponemal antibody. The FTA-ABS and TPIA tests both use *T. pallidum* as antigen, but less correlation than expected was seen. Since the antibody for the FTA-ABS test used in these experiments was anti-Ig the antibody detected in the test was mainly the IgG antibody. In the TPIA test, however, the sensitivity to IgM antibody was much higher than that to IgG antibody, as was apparent from the results of gel filtration (Figure 3). This may be the main reason for the lack of correlation between the two tests.

The results of the qualitative TPIA test showed a high correlation with those of the TPHA test, presumably because treponemal antigen was used. In the quantitative TPIA test, however, there were

differences in the distribution of the antibody titre and correlation was low (Figure 2). This may be due to the following reasons:

- (1) As reported by Robertson *et al* (1975) it was clear from the gel filtration that antibody detected by the TPHA test was mainly in the IgG fraction but antibodies detected by the TPIA test were IgM;
- (2) Unlike the TPHA test the TPIA test requires complement.

In conclusion, the TPIA test differs considerably from the glass plate, TPHA, and FTA-ABS-IgG tests, especially in its reactivity in the IgM fraction. From the results of the tests using the sera of untreated patients it was clear that the TPIA test had a high sensitivity to the antibody, which appeared soon after infection. In the instance of Cases 1 and 5, only the TPIA test and TPIA and FTA-ABS tests respectively gave positive results (Table 2). Case 22 was examined just before symptoms occurred when serum antibodies were absent, but after 10 days the glass plate and TPIA tests gave positive results. At the end of the first month of treatment the index decreased to the range of doubtful-positive, and the glass plate test gave negative results. The results of the TPHA test remained negative. Since sera from Cases 20 and 21 showed positive results only to the glass plate test, these were considered to be biological false-positive results. Sera from Cases 20 and 21, however, were retested after nine and 14 days respectively and showed doubtful-positive and positive results in the TPIA and FTA-ABS tests respectively. These cases were considered to be at the seronegative stage. From the above results it can also be assumed that the TPIA and other serological tests differ considerably even though they both depend on reactions using treponemal antigen.

The authors wish to thank Dr Kawashima of the Metropolitan Okubo Hospital and Dr Onoda of the Metropolitan Taito Hospital for their donation of the test specimens and their clinical advice. This work was supported by a research grant from the Waxmann Foundation.

References

- Miller, F., and Metzger, H. (1965). Characterization of a human macroglobulin. II. Distribution of the disulfide bonds. *Journal of Biological Chemistry*, 240, 4740-4745.
- Mizuoka, K., Mitsuhashi, T., Fukuoka, Y. and Kawamura, A. (1973). Review of serologic tests for syphilis and treponemal antibody test now in use in Japan. In *Immunopathology of the Skin, Labelled Antibody Studies*, p. 417. Douden, Hutchinson and Ross Inc: Stroudsburg, Pennsylvania, USA.

TPIA test for serodiagnosis of syphilis

- Nelson, R. A. jun. (1953). The immune-adherence phenomenon. An immunologically specific reaction between microorganisms and erythrocytes leading to enhanced phagocytosis. *Science*, **118**, 733–737.
- Olansky, S., Harris, A., and Casey, H. (1954). Immune-adherence test for syphilis. Comparison with TPI and VDRL slide tests. *Public Health Reports*, **69**, 521–526.
- Robertson, D. H. H., McMillan, A., Young, H., and Henrichsen, C. (1975). Clinical value of the *Treponema pallidum* haemagglutination test. *British Journal of Venereal Diseases*, **51**, 79–82.
- Tanaka, S., Suzuki, T., and Numata, T. (1978). TPIA test for serodiagnosis of syphilis. I. An improved method of TPIA test. *British Journal of Venereal Diseases*, **54**, 369–373.