Evaluation of the Passive Particle Agglutination Test in the Serodiagnosis and Follow-up of Syphilis

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Key Words: Syphilis serodiagnosis; Passive particle agglutination test; TP-PA and syphilis; Syphilis follow-up

Abstract

We performed the present study to determine the rate of concordance of the fluorescent treponemal antibody absorption test (FTA-ABS) and of the microhemagglutination assay for antibodies to Treponema pallidum (MHA-TP) with the passive particle agglutination test (TP-PA) in patients with early syphilis and to observe the reactivity of the rapid plasma reagin (RPR), MHA-TP, and the TP-PA tests for 1 year after therapy. The study included 449 people who were given therapy if they had syphilis and followed up for 1 year. The rate of concordance of the TP-PA with the MHA-TP was 98.4%, and it was 98.9% with the FTA-ABS. During follow-up, a significant decrease of antibodies was found in 56%, 26%, and 70% of the patients when using the RPR, the MHA-TP, and the TP-PA, respectively. The TP-PA seems to be an adequate routine assay for the diagnosis of syphilis, being as sensitive as the FTA-ABS test in primary syphilis and as useful as the RPR test in monitoring therapy.

Syphilis is a systemic infection due to Treponema pallidum subsp. pallidum, with clinical manifestations that may1 mimic many other diseases. Laboratory diagnosis is, therefore, of great importance, but because there is no culture medium available for T. pallidum, and polymerase chain reaction is still being improved and not available for the majority of syphilis stages,2,3 one has to rely on microscopy and serologic tests. Since microscopy can be used only for specimens from skin or mucosal lesions, the laboratory diagnosis of infection is generally performed by screening serum with a nontreponemal test, such as the rapid plasma reagin test (RPR), and confirming with a treponemal test, which uses treponemal antigen.2 Although many of these tests have been developed over the years, modifications of the fluorescent treponemal antibody (FTA) test and of the T. pallidum hemagglutination test, respectively the FTA absorbent (FTA-ABS) and the microhemagglutination assay (MHA-TP),4 remain the most frequently used tests.

The nontreponemal test titers decrease with adequate therapy and with time and generally become nonreactive.5,6 The tests that use treponemal antigens may remain reactive forever and, thus, should not be used to monitor adequacy of therapy.7 The most widely used of the specific tests is the MHA-TP, because it is easier to perform and quicker to read and does not require as much equipment or trained staff as the fluorescent test. However, the FTA-ABS provides higher sensitivity in primary syphilis.8,9

The passive particle agglutination test (TP-PA) is a specific agglutination test similar to the MHA-TP that uses gelatin particles instead of erythrocytes10 and also is easy and quick to perform. However, its titers seem to decline with therapy, like a nonspecific test, and to be as sensitive as the FTA-ABS in primary syphilis.
We compared the most used specific tests (MHA-TP and FTA-ABS) and the TP.PA at the time of enrollment, to determine whether this specific test could be used as an alternative to the MHA-TP and the FTA-ABS tests. We also tested the reactivity of the RPR, the MHA-TP, and the TP.PA, repeatedly performed until 6 and 12 months after adequate therapy, to determine whether the tests were able to reflect the efficiency of therapy.

Materials and Methods

We studied 449 patients suspected of having early syphilis who were attending a sexually transmitted disease clinic or were admitted to an infectious disease unit in a hospital in the Lisbon, Portugal, area. Blood was obtained for serologic tests (RPR, MHA-TP, FTA-ABS, and TP.PA), and patients were given standard therapy. The scientific council of the Instituto de Higiene e Medicina Tropical approved the study, since it represents the research committee on human subjects.

Patients were asked to return at 1, 2, 3, 6, and 12 months after therapy. Only 54 patients returned for follow-up. A questionnaire to exclude the possibility of reinfection, clinical examination, and collection of blood were performed at each visit.

All serologic tests (RPR Macro-Vue Becton Dickinson, Franklin Lakes, NJ; MHA-TP, Phasyl-Diagast, Lille Cedex, France; Serodia-T.P. PA, Fujirebio, Tokyo, Japan; FTA-ABS, EUROIMMUN, Seekamp, Germany) were performed according to established methods, and persons performing the tests were blinded to the results of each test when running the other tests on the same patient specimen.

Results

Concordance of Specific Test Results at Enrollment

Of the 449 serum samples studied, 324 (72.2%) were reactive in the MHA-TP, 331 (73.7%) in the TP.PA, and 336 (74.8%) in the FTA-ABS at the time of enrollment. Nonreactive samples were as follows: 125 (27.8%) in the MHA-TP, 118 (26.3%) in the TP.PA, and 113 (25.2%) in the FTA-ABS. When comparing the TP.PA with the MHA-TP, the TP.PA showed a sensitivity of 100.0% and a specificity of 94.4%. Comparison of the TP.PA with the FTA-ABS showed a sensitivity of 98.5% and a specificity of 100.0%. The rates of concordance were 98.4% and 98.9% when comparing the TP.PA with the MHA-TP and the FTA-ABS, respectively.

Follow-up

The great majority of patients who did not return for follow-up had clinical and serologic profiles compatible with latent syphilis. Of the 44 patients who were evaluated serologically at least during the 6 months after therapy, 10 had primary syphilis, 13 had secondary syphilis, and 31 were in the latent stage. At that time, none of the patients had clinical signs of syphilis.

A substantial decrease of antibodies (at least 2-fold dilution) in the RPR after 6 months following therapy was found in 30 (56%) of 54 patients. The same was found in 14 (26%) of 54 and in 38 (70%) of 54 patients when using the MHA-TP and the TP.PA, respectively.

Seroreversion was observed in 7 (13%) of 54 patients. Seroreversion occurred in 2 patients after 6 months and in 5 patients after 12 months, but it was evident only in the nontreponemal test.

Patients With Primary Syphilis

From the 10 patients clinically diagnosed as having primary syphilis, 10 were observed until 6 months after

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<table>
<thead>
<tr>
<th>MHA-TP</th>
<th>Reactive</th>
<th>Nonreactive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP.PA Reactive</td>
<td>324</td>
<td>7</td>
<td>331</td>
</tr>
<tr>
<td>TP.PA Nonreactive</td>
<td>0</td>
<td>118</td>
<td>118</td>
</tr>
<tr>
<td>TP.PA Total</td>
<td>324</td>
<td>125</td>
<td>449</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FTA-ABS</th>
<th>Reactive</th>
<th>Nonreactive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP.PA Reactive</td>
<td>331</td>
<td>0</td>
<td>331</td>
</tr>
<tr>
<td>TP.PA Nonreactive</td>
<td>5</td>
<td>113</td>
<td>118</td>
</tr>
<tr>
<td>TP.PA Total</td>
<td>336</td>
<td>113</td>
<td>449</td>
</tr>
</tbody>
</table>

MHA-TP, microhemagglutination assay for antibodies to Treponema pallidum; TP.PA, passive particle agglutination test.

* Sensitivity (324/324), 100.0%; specificity (118/125), 94.4%; agreement (442/449), 98.4%.

* Sensitivity (331/336), 98.5%; specificity (113/113), 100.0%; agreement (444/449), 98.9%.
enrollment and 4 until 12 months after enrollment Table 3. At 6 months, 8 had at least a 2-fold decrease in the RPR titer, 3 in the MHA-TP titer, and 9 in the TP.PA titer. All patients observed until 1 year after therapy had at least a 2-fold decrease in the RPR and TP.PA titers, while titers were maintained in the MHA-TP (Table 3), and 2 patients experienced seroreversion that was evident in the RPR.

Table 3
Follow-up of Patients With Primary Syphilis at 6 and 12 Months After Therapy*

<table>
<thead>
<tr>
<th></th>
<th>RPR</th>
<th>MHA-TP</th>
<th>TP.PA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 mo</td>
<td>12 mo</td>
<td>6 mo</td>
</tr>
<tr>
<td>No decrease in titer</td>
<td>2 (20)</td>
<td>0 (0)</td>
<td>7 (70)</td>
</tr>
<tr>
<td>Decrease in titer</td>
<td>8 (80)</td>
<td>2 (50)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Seroreversion</td>
<td>0 (0)</td>
<td>2 (50)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

MHA-TP, microhemagglutination assay for antibodies to Treponema pallidum; RPR, rapid plasma reagin; TP.PA, passive particle agglutination test.

* Data are given as number (percentage).

Table 4
Follow-up of Patients With Secondary Syphilis at 6 and 12 Months After Therapy*

<table>
<thead>
<tr>
<th></th>
<th>RPR</th>
<th>MHA-TP</th>
<th>TP.PA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 mo</td>
<td>12 mo</td>
<td>6 mo</td>
</tr>
<tr>
<td>No decrease in titer</td>
<td>3 (23)</td>
<td>0 (0)</td>
<td>8 (62)</td>
</tr>
<tr>
<td>Decrease in titer</td>
<td>10 (77)</td>
<td>4 (67)</td>
<td>5 (38)</td>
</tr>
<tr>
<td>Seroreversion</td>
<td>0 (0)</td>
<td>2 (33)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>6</td>
<td>13</td>
</tr>
</tbody>
</table>

MHA-TP, microhemagglutination assay for antibodies to Treponema pallidum; RPR, rapid plasma reagin; TP.PA, passive particle agglutination test.

* Data are given as number (percentage).

Table 5
Follow-up of Patients With Latent Syphilis at 6 and 12 Months After Therapy*

<table>
<thead>
<tr>
<th></th>
<th>RPR</th>
<th>MHA-TP</th>
<th>TP.PA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 mo</td>
<td>12 mo</td>
<td>6 mo</td>
</tr>
<tr>
<td>No decrease in titer</td>
<td>17 (55)</td>
<td>2 (17)</td>
<td>25 (81)</td>
</tr>
<tr>
<td>Decrease in titer</td>
<td>12 (39)</td>
<td>9 (75)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>Seroreversion</td>
<td>2 (6)</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>12</td>
<td>31</td>
</tr>
</tbody>
</table>

MHA-TP, microhemagglutination assay for antibodies to Treponema pallidum; RPR, rapid plasma reagin; TP.PA, passive particle agglutination test.

* Data are given as number (percentage).

Patients With Secondary Syphilis
Secondary syphilis was diagnosed in 13 patients followed up until 6 months after therapy; 6 of these patients were monitored until 12 months Table 4. At 6 months, 10 had at least a 2-fold decrease in the RPR titer, 5 in the MHA-TP, and 11 in the TP.PA, while 3 had the same titer in the RPR, 8 in the MHA-TP, and 2 in the TP.PA. Every patient seen at 12 months after therapy had a decrease of at least 2-fold in the RPR and TP.PA titers, while 2 maintained the initial titer in the MHA-TP.

Patients With Latent Syphilis
Of the patients with latent syphilis, 31 were followed up during 6 months and 12 until 12 months after therapy Table 5. At 6 months, there was no substantial decrease in the RPR titer in 17 patients, there was at least a 2-fold decrease in 12, and seroreversion in 2. With the MHA-TP, there was no substantial decrease in titer in 25 patients and at least a 2-fold decrease was observed in 6, and there was maintenance of the TP.PA titer in 13 and a decrease in 18. In the patients followed up until 12 months after therapy, seroreversion was observed in another patient by the RPR result, and there was at least a 2-fold decrease in the RPR titer in 9, in 5 with the MHA-TP, and in 9 with the TP.PA.
In 2 patients, the titer was maintained with RPR, in 7 it was maintained with the MHA-TP, and in 3 it was maintained with the TP.PA.

Discussion

The TP.PA is an agglutination test for syphilis that differs from the MHA-TP because it uses an artificial carrier made of soluble antigen and gum arabic12 instead of erythrocytes. Like the FTA-ABS, it seems to be more sensitive than the MHA-TP and the RPR in primary syphilis.13,14 However, unlike the FTA-ABS, it does not need a fluorescent microscope to be observed. It also is easier to read than the MHA-TP and is not subject to heterophil reactions.15 Another advantage of this test would be that, unlike other treponemal tests, it could serve as an indication of successful treatment, since TP.PA titers decrease with therapy.

In the present study, we evaluated the sensitivity and the specificity of the TP.PA, comparing it with the most commonly used specific tests for the diagnosis of syphilis, the MHA-TP and the FTA-ABS. The results obtained showed high sensitivity and specificity of the TP.PA when compared with each of the other specific tests. Furthermore, the rate of concordance of the TP.PA with the MHA-TP was 98.4%, while with the FTA-ABS it was 98.9%. This is in accordance with the findings of other investigators, who found that when comparing any 2 treponemal tests, the agreement was 97%.15

As in other studies,16,17 we also have shown that the FTA-ABS is more sensitive than the MHA-TP in primary syphilis cases, and it seems that the same could be applied to the TP.PA.

We also compared the TP.PA titers after treatment with those of the RPR and the MHA-TP, in view of verifying whether the TP.PA could be used to monitor therapy. This was done in a small number of patients (those who returned for follow-up), since most of them are from high-risk groups for sexually transmitted diseases. People at high risk for sexually transmitted diseases commonly do not return for consultation, especially when they feel healthy. We found that for primary syphilis and after therapy, a higher number of patients had a significant decrease in RPR and TP.PA titers than in the MHA-TP. The same happened in secondary and especially in latent syphilis, where these differences were more frequently observed. Although numbers were small, at least a 2-fold decrease in titers seems to be seen more often with the TP.PA than with the RPR. In the present study, and as other authors have found,18,19 seroreversion was observed only in a few cases, mostly by the end of 12 months, and it was evident only by the RPR test.

Conclusions

Our findings suggest that the TP.PA is adequate to be used as a routine assay for the diagnosis of syphilis, being as sensitive as the FTA-ABS test in primary syphilis and as useful as the RPR test in monitoring therapy. It also has the advantages of not requiring a fluorescent microscope, being less subjective than the FTA-ABS and easier to read than the MHA-TP.

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References


