Evaluation of the *Treponema pallidum* Particle Agglutination Technique (TP.PA) in the Diagnosis of Neurosyphilis

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The *Treponema pallidum* particle agglutination technique (TP.PA) was evaluated, in comparison with the Venereal Disease Research Laboratory (VDRL) test, microhemagglutination assay for *Treponema pallidum* antibodies (MHA-TP), and fluorescent treponemal antibody-Abs (FTA-Abs) test for the diagnosis of neurosyphilis. We have studied 198 cerebrospinal fluid (CSF) samples from patients with syphilis, including neurosyphilis, treated syphilis, and with other neurological manifestations than neurosyphilis. All tests were nonreactive in these last group of patients. In the neurosyphilis patients, sensitivity of the TP.PA was 100%. The performance of this

test in CSF from patients with primary syphilis was as good as that of the other tests. In secondary and latent syphilis, the TP.PA results (27 reactive samples/73) were similar to those of the MHA-TP (25 reactive samples/73). In the individuals treated for syphilis, the TP.PA, FTA-Abs, and MHA-TP tests were found to be reactive in eight, six, and eight samples, respectively. In conclusion, it seems that the TP.PA can be used in CSF to diagnose neurosyphilis, although as for other serological tests, interpretation of results should be done in conjunction with other neurosyphilis parameters. J. Clin. Lab. Anal. 20:233–238, 2006. © 2006 Wiley-Liss, Inc.

Key words: syphilis; laboratory diagnosis; CSF

INTRODUCTION

During the last few years, syphilis has increased all over the world (1). *Treponema pallidum* may invade the central nervous system early in the course of infection (2,3) and is apparently independent of human immunodeficiency virus (HIV) infection (3). However, several studies suggest that in HIV patients, unusual presentations of syphilis (4,5) and rapid progressive course for neurosyphilis are common (2,6–12). Furthermore, the diagnosis of neurosyphilis is difficult and it becomes worse in HIV patients (10,11,13).

Failure of penicillin therapy to prevent neurosyphilis has also been reported in HIV patients (14,15), leading investigators to believe that HIV may increase the risk for treatment failure. In view of this situation, recommendations have changed in the last years: it has been recommended that all patients with syphilis should be

screened for HIV (2,16,17), that only HIV patients with syphilis should have a cerebrospinal fluid (CSF) examination for *T. pallidum* infection (2,6), and that this must be done in all individuals with late or indeterminate latent syphilis (18).

Although a “gold standard” for the diagnosis of neurosyphilis is not available (18,19), a reactive serum treponemal test and a reactive Venereal Disease Research Laboratory (VDRL) CSF test are generally considered definitive evidence of neurosyphilis (6,18,20); however, false-negative and rarely false-positive results

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may occur (18,21). On the other hand, cerebrospinal leukocytosis and elevated protein concentration are nonspecific findings and therefore considered as presumptive diagnosis (18,21). Some authors suggest that both the fluorescent treponemal antibody test (FTA-Abs) and the *T. pallidum* microhemagglutination test (MHA-TP) are more sensitive, although less specific than the VDRL test (21–23) in the diagnosis of neurosyphilis, but this is not accepted by everyone (24,25). It is also believed that a nonreactive result in the FTA-Abs CSF test (21,23,26) could be used to rule out neurosyphilis. Because of the possibility of CSF contamination some authors recommended measuring the immunoglobulin G (IgG) and IGM index, the albumin quotient, or the *T. pallidum* hemagglutination assay (TPHA) index, but this is rather time consuming and difficult to implement in daily laboratory routine.

Therefore, the diagnosis of neurosyphilis continues to be a problem and usually relies on various combinations of reactive serological tests results, alterations of cell count or proteins in the CSF, or clinical manifestations.

The *T. pallidum* particle agglutination test (TP.PA) is a specific test that uses gelatin particles and has proven to be an appropriate alternative to other validated specific tests (27,28) in sera. Until now, the TP.PA has not been recommended for use in the CSF and to our knowledge no studies have been performed on the usefulness of this test in the diagnosis of neurosyphilis. We investigated CSF from patients suspected of having neurosyphilis, both symptomatic and asymptomatic, and negative controls with nonreactive sera by the VDRL test, MHA-TP, and FTA-Abs results, evaluating whether the TP.PA could be an alternative to other tests in the laboratory diagnosis of neurosyphilis.

MATERIALS AND METHODS

During 1999–2002, sera and CSF samples from individuals admitted to two hospitals in the Lisbon area were obtained. A total of 152 patients had reactive serological tests for syphilis, from which 133 were infected with HIV and 19 were not infected with HIV; one of those patients had symptomatic neurosyphilis and the remaining latent syphilis. A total of 46 had other neurological infections (Table 1) than neurosyphilis and nonreactive serological tests (VDRL, MHA-TP, FTA-abs) for syphilis, 33 of these patients had antibodies against HIV.

Patients with antibodies against *T. pallidum* (152 patients) were divided as follows: 16 presented with the diagnosis of neurosyphilis, 7 of the 16 had symptomatic neurosyphilis: clinical manifestations and laboratory criteria for neurosyphilis (positive MHA-TP and/or FTA-Abs tests, increased number of mononuclear cells

TABLE 1. Neurological infections from patients without syphilis

HIV+vo	Number	HIV–vo	Number
Toxoplasma encephalitis	8	Guillan-Barré syndrome	1
Cryptococcal meningitis	7	Viral encephalitis	3
Tuberculosis of CNS	5	Aseptic meningitis	6
Primary lymphoma	1	Septic meningitis	3
Viral encephalitis	10		
Leukoencephalitis	2		

+vo, positive; –vo, negative.

>10/mm³ (white blood cells [WBC]), plus reactive VDRL test in the CSF, according to Goh and van Voorst Vader (29)); and 9 of the 16 presented with asymptomatic neurosyphilis: laboratory criteria of neurosyphilis and no clinical signs or symptoms of central nervous system involvement. Two other individuals had clinical signs and symptoms of neurosyphilis: reactive TP.PA and FTA-Abs, increased cell count, and nonreactive VDRL. The remaining 134 had no neurological symptoms or signs of neurosyphilis. From these, four had primary syphilis (primary chancre and lymphadenopathy), six presented with secondary syphilis (generalized nonitchy polymorphic rash affecting the palms and soles and reactive serological tests), in 67 latent syphilis was diagnosed (asymptomatic patients with reactive serology for syphilis), and 57 had previously treated syphilis.

The scientific council of the Instituto Higiene e Medicina Tropical approved the study, since it represents the research committee on human subjects.

All samples were tested by the VDRL (VDRL-IMMUNOTREP Omega Diagnostic Limited, Scotland, UK), MHA-TP (Phasyl 210 Diasorin-Diagast, Loos, France), FTA-Abs *T. pallidum* (EUROIMMUN; Medizinische Laboratorien AG, Luebeck, Germany), and TP.PA (Serodia-TP.PA, Fujirebio Inc., Tokyo, Japan) tests, according to manufacturers instructions. All reactive samples were titrated by serial dilutions. CSF WBC were considered increased if ≥ 10 WBCs/ μ L. CSF samples were considered contaminated with blood and discarded whenever red cell count >0.001% (30–32).

Persons performing the tests were blinded to the results of each test when running the other tests in the same patient specimen and all patients considered to be infected with *T. pallidum* were given standard therapy.

RESULTS

All CSF samples, 46 from individuals with other neurological infections than neurosyphilis and nonreactive sera were nonreactive in all serological tests.

All CSF samples from the four patients with primary syphilis had normal cell count and nonreactive syphilis serological tests in the CSF. Of the six samples from patients with secondary syphilis, four were nonreactive in all serological tests, two of which had increased cell count. The remaining two samples only had reactivity in the MHA-TP and in the TP.PA tests (Table 2).

The TP.PA was reactive in all 16 CSF samples from patients with symptomatic and asymptomatic neurosyphilis. Therefore, the sensitivity of the TP.PA in this stage of syphilis was 100%. The two samples from patients presenting with clinical and laboratory criteria

of neurosyphilis, with the exception of a reactive VDRL, had reactive TP.PA tests (Table 3).

A total of 67 CSF samples were from patients with latent syphilis (Table 4). From these, 38 had nonreactive serological tests, including the TP.PA, four of which had increased cell count. In two samples, only the MHA-TP was found to be reactive and in another one the MHA-TP showed reactivity and its cell count was increased. One CSF sample was reactive only for the VDRL test and had increased cell count. The remaining 25 CSF samples from patients with latent syphilis presented reactivity in the TP.PA test. In four of them, only this

TABLE 2. Cell count and serological tests in CSF samples from individuals with primary and secondary syphilis

	Cell count	VDRL	MHA-TP	TP.PA	FTA-Abs	Number
Primary syphilis	Normal	—	—	—	—	4
Secondary syphilis	Normal	—	—	—	—	2
	>	—	—	—	—	2
	Normal	—	+	+	—	2

—, non-reactive; +, reactive; >, increased.

TABLE 3. Cell count and serological tests in CSF samples from patients with neurosyphilis

	Cell count	VDRL	MHA-TP	TP.PA	FTA-Abs	Number
Symptomatic neurosyphilis	>	+	+	+	+	6
	>	+	—	+	+	1
Asymptomatic neurosyphilis	>	+	+	+	+	9
Clinical and laboratory criteria of neurosyphilis and nonreactive VDRL	>	—	+	+	+	2

—, non-reactive; +, reactive; >, increased.

TABLE 4. Cell count and serological tests in CSF samples from individuals with latent syphilis

Cell count	CSF tests				Number
	VDRL	MHA-TP	TP.PA	FTA-Abs	
Normal	—	—	—	—	34
>	—	—	—	—	4
Normal	—	+	—	—	2
>	—	+	—	—	1
>	+	—	—	—	1
Normal	—	—	+	—	4
Normal	—	+	+	—	4
>	—	+	+	—	1
Normal	—	+	+	+	7
>	—	+	+	+	7
Normal	+	+	+	+	1
>	—	—	+	Indeterminate	1
Total					67

—, non reactive; +, reactive; >, increased.

TABLE 5. Cell count and serological tests in CSF samples from individuals with previously treated syphilis

Cell count	CSF tests				Number
	VDRL	MHA-TP	TP.PA	FTA-Abs	
Normal	—	—	—	—	47
Normal	+	—	—	—	1
Normal	—	—	—	+	1
Normal	—	+	+	—	3
Normal	—	+	+	+	4
Normal	+	+	+	Indeterminate	1
Total					57

—, non-reactive; +, reactive.

test showed reactivity, in another five the MHA-TP was also reactive, with one of these presenting increased cell count. A total of 14 had reactivity in all treponemal tests, seven with increased cell count and the other seven having normal cell count. One patient presented with a reactive CSF sample in all serological tests and a normal cell count, while the last patient had increased cell count and indeterminate FTA-Abs.

We also examined the results obtained in the 57 spinal fluid samples from patients who had been treated for syphilis (Table 5). In 47 of them, all parameters studied were normal. In the majority (seven) of the remaining 10 patients, the TP.PA results were in accordance with those of the other parameters studied.

DISCUSSION AND CONCLUSIONS

The diagnosis of neurosyphilis is still a problem. Although a number of tests to detect *T. pallidum* are presently available, those that can be used in the diagnosis of this infection are in a smaller number and interpretation of results is frequently difficult. For example, the VDRL test seems to be very specific, originating no false-positive results, but seems to be insensitive (18,21,30,33,34). Therefore, and in accordance with some authors, a reactive test is indicative of neurosyphilis, but a negative VDRL does not exclude this diagnosis (18,22,24). On the contrary, the FTA-Abs test seems to be highly sensitive, serving to rule out CSF infection, since it seems to be nonreactive in all samples from patients who do not have neurosyphilis (23,34). However, the evaluation of tests to be used for the diagnosis of neurosyphilis continues to be difficult and challenging, because a “gold standard” test is still not available and although isolation of *T. pallidum* is sensitive during early invasion of the central nervous system (3), it is impracticable in the routine laboratory.

In this study, we assessed the performance of the TP.PA test in the CSF and compared it with other treponemal tests in the various stages of syphilis.

In four patients with primary syphilis, the TP.PA was in agreement with the other serological tests. In two samples from the six patients with secondary syphilis, only the TP.PA and MHA-TP tests were reactive in the CSF, which could probably be considered false positive results.

In the group of neurosyphilis patients (symptomatic and asymptomatic), the sensitivity of the TP.PA test was 100%.

The TP.PA test was also reactive in the CSF from two individuals who had all criteria for the diagnosis of symptomatic neurosyphilis, with the exception of the VDRL test. We could not consider these patients to have neurosyphilis, since the criteria used in this study included a reactive VDRL test. However, in view of some authors who state that a nonreactive VDRL test does not exclude neurosyphilis (18,21,22), it seems that these could be false-negative VDRL results. Although the criteria for the diagnosis of neurosyphilis used by Larsen et al. (21) were different from those of our study, they have described a sensitivity of 90% and 10% for the VDRL test, respectively, in patients with symptomatic and asymptomatic neurosyphilis.

In 42 individuals from the latent syphilis group, the TP.PA technique and all other serological tests were nonreactive, with the exception of four. One of these last patients had a reactive VDRL test, which is considered diagnostic of neurosyphilis (18,19). However, it has also been said that a nonreactive FTA-Abs excludes a diagnosis of neurosyphilis (19,32). Therefore, we cannot make definitive conclusions about the presence of neurosyphilis in this patient.

When we analyzed the 25 samples from patients with latent syphilis with reactive TP.PA, this technique was mostly in agreement with the MHA-TP, although the FTA-Abs was also reactive in six. It is difficult to evaluate the performance of this technique in one of these patients, since his CSF sample showed an indeterminate FTA-Abs test and increased cell count. This is once again a case in which the diagnosis of

neurosyphilis cannot be made with certainty, but it can not be ruled out.

In relation to the 57 patients with treated syphilis, the TP.PA test was in agreement with the other serological tests in 48, being nonreactive in 47 and reactive in one sample, in which the FTA-Abs test was indeterminate. In the remaining nine samples, the TP.PA test agreed mostly with the MHA-TP and in four with the FTA-Abs. Actually, one should note that in this study, all CSF samples from patients with treated syphilis had normal cell count, which could signify that, after treatment of the patients, CSF cells decreased, but specific antibodies still circulate in the CSF. This is in accordance with an article by Marra et al. (35), in which patients treated for neurosyphilis had normalized CSF cell count 12.4 months after completion of therapy, while the CSF VDRL test still showed reactivity in 6% of the patients 14.2 months after treatment.

The results of this study seem to suggest that the performance of the TP.PA in CSF samples is similar to other serological tests in the diagnosis of neurosyphilis, specially to the MHA-TP. Since the TP.PA is easier to read and more stable than the MHA-TP, does not need a fluorescent microscope, and is less subjective than the FTA-Abs, it can be easily adapted to laboratory routine. However, as for the other serological tests, and as previously stated by other authors (33,36), interpretation of results should be done carefully, in conjunction with biochemical examination of the CSF and clinical signs and symptoms of neurosyphilis.

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