Nontreponemal Tests in the Diagnosis of Neurosyphilis: An Evaluation of the Venereal Disease Research Laboratory (VDRL) and the Rapid Plasma Reagin (RPR) Tests

Rita Castro,* Emília Simões Prieto, and Filomena da Luz Martins Pereira
*Instituto de Higiene e Medicina Tropical, Unidade de Doenças Sexualmente Transmitidas,
Lisboa, Portugal*

The Venereal Disease Research Laboratory (VDRL) test has long been considered the best serological test for the diagnosis of neurosyphilis. The goal of this study was to find out if the Rapid Plasma Reagin (RPR) could be an alternative to the VDRL. Cerebrospinal fluid (CSF) and sera samples from patients in the following stages of syphilis were tested: 8 had symptomatic and 16 asymptomatic neurosyphilis, 4 were in the primary stage, 6 had secondary syphilis, and 92 were in the latent stage. We have also studied 61 samples from individuals with treated syphilis and 126

with other neurological diseases than neurosyphilis. All the CSF samples were studied with both RPR and VDRL tests. RPR and VDRL test results were mostly concordant. The specificity of these tests for current neurosyphilis was 99% for the VDRL and 99.3% for the RPR, whereas the sensitivity was 70.8 and 75%, respectively, for the VDRL and RPR. In view of these results it seems to us that the RPR could be an alternative to the VDRL in the diagnosis of neurosyphilis. *J. Clin. Lab. Anal.* 22:257–261, 2008. © 2008 Wiley-Liss, Inc.

Key words: neurosyphilis; RPR; VDRL; CSF

INTRODUCTION

Cerebrospinal fluid (CSF) examination is essential in the diagnosis of neurosyphilis, with serological testing of the CSF playing a major role.

In 1985, Larsen et al. (1) found that the Venereal Disease Research Laboratory (VDRL) had a higher specificity and sensitivity than the Rapid Plasma Reagin (RPR) test. Although no other studies were published comparing these two tests, the low specificity of the RPR in patients with other possible neurologic diseases described in Larsen et al. study led to the assumption that the RPR should never be used to diagnose neurosyphilis. Furthermore, studies evaluating the performance of serological tests or polymerase chain reaction techniques in the CSF almost always used the VDRL as the reference test (2–4).

The goal of this study was to determine if the RPR could be an alternative to the VDRL in the diagnosis of neurosyphilis.

MATERIALS AND METHODS

Three hundred and fourteen (314) sera and CSF from patients suspected of having syphilis or other neurological

diseases were studied. The characteristics of these individuals are reported in Table 1. In 187 serological tests for syphilis were reactive (RPR and/or microhemagglutination assay for *Treponema pallidum* (MHA-TP)), from whom 126 were not treated and 61 had previously been treated, whereas 126 had other neurological diseases than neurosyphilis and nonreactive blood serological tests (RPR and MHA-TP).

As the RPR was being evaluated as an alternative to the VDRL, this test could not be used as the only criterion, as recommended by the Centers for Disease Control and Prevention (CDC) (5), or as one of the criteria for the diagnosis of neurosyphilis, as recommended by Goh and van Voorst Vader (6). Our criteria of neurosyphilis included all CSF samples from individuals with reactive serological tests for syphilis in sera (RPR $\geq$ 1:8 and MHA-TP $\geq$ 1:80) and with a reactive

*Correspondence to: Rita Castro, Instituto de Higiene e Medicina Tropical, Unidade de Doenças Sexualmente Transmitidas, Rua da Junqueira, No. 96, 1349-008 Lisboa, Portugal.
E-mail: ritacastro@ihmt.unl.pt

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CSF-Fluorescent Treponemal Antibody Absorption Assay (CSF-FTA-Abs), increased proteins, and cell count. According to these criteria, 24 patients were considered to have neurosyphilis, from which 8 had symptomatic neurosyphilis (disease meningo-vascular—6, meningitis—1, cranial neuritis—1) and 16 asymptomatic neurosyphilis (no neurologic symptoms or signs). There was, however, 1 patient with all our clinical and laboratory criteria of neurosyphilis, with the exception of increased proteins. All of these 25 patients were HIV positive (Table 1) with the exception of 1 with meningo-vascular neurosyphilis.

The other 163 patients with reactive blood serological tests and with no neurologic symptoms or signs were classified in the following stages: 4 in primary syphilis (primary chancre and lymphadenopathy) who were all infected with HIV; 6 in secondary syphilis (generalized nonitchy polymorphic rash affecting the palms and soles and reactive serological tests) 5 being coinfecting with HIV; 92 in the latent stage (asymptomatic patients who have not been treated with reactive RPR and MHA-TP), including 86 coinfecting with HIV; and 61 had previously been treated for syphilis (patients who after treatment had sustained decreased RPR titers and reactive MHA-TP or only a reactive MHA-TP) from which 58 were also infected with HIV (Table 1).

The Scientific Council of the Instituto de Higiene e Medicina Tropical approved the study, as it represents the research committee on human subjects.

All sera samples were tested by RPR (Macro-Vue™ “RPR Card Antigen Suspension,” Lovetone Circle Sparks, MD) and FTA-Abs *T. pallidum* (EUROIMMUN, Medizinische Laboratorien AG, Luebeck, Germany) and all CSF samples by the VDRL (VDRL-IMMUNOTREP—Omega Diagnostic Limited, Scotland, UK), RPR, and FTA-Abs *T. pallidum*,

according to manufacturers' instructions. All reactive samples were titrated by serial dilutions. CSF white blood cells and proteins were considered high if ≥ 10 cell/mm³ and ≥ 45 mg/dl, respectively. CSF samples were considered contaminated with blood and discarded whenever red cell count was >0.001 /mm³ (7–9).

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 with syphilis were given appropriate therapy.

RESULTS

Results of serological tests in samples from patients (126) with other neurological disorders than syphilis are described in Table 2.

All CSF tests were nonreactive in all samples, with the exception of one specimen, from an HIV-negative patient with the diagnosis of hydrocephaly, in which the VDRL and RPR were only reactive at a titer of 1:1, cell count was normal (6 cell/mm³), and proteins were high (75 mg/dl).

Table 3 contains the results of the CSF laboratory parameters performed in this study from patients with primary and secondary syphilis.

All serological tests were nonreactive in all of these patients. Cell count and proteins were also normal in every patient with primary syphilis. In relation to samples from individuals with secondary syphilis, three had no alterations of the cell count and proteins, one had both cell count and proteins elevated, in another only the cell count was elevated, and in the last one only proteins were increased.

Eight CSF samples from patients with symptomatic neurosyphilis and 16 with asymptomatic neurosyphilis were analyzed (Table 4).

In seven of the eight patients with symptomatic neurosyphilis, the VDRL and the RPR were both also reactive, whereas in one sample only the VDRL was nonreactive. In the asymptomatic group, nine individuals presented reactivity in both the RPR and the VDRL in their CSF samples, in one only the RPR was nonreactive, in four both the VDRL and the RPR were nonreactive, and in two only the VDRL was nonreactive.

There was one sample from a patient with symptoms and signs of neurosyphilis in which the VDRL was nonreactive, proteins were normal, but the other parameters showed alterations.

In 69 of the 92 CSF samples from patients with latent syphilis (Table 5), all parameters studied were normal. In seven, only the FTA-Abs test was reactive and in one all serological tests were reactive, but cell count and proteins were normal.

Three other CSF samples presented normal cell count, high proteins, reactive FTA-Abs, and nonreactive

TABLE 1. Characterization of the Individuals Included in the Study, in Relation to their Syphilis and HIV Status

	HIV +ve ^a	HIV –ve ^b
With syphilis		
Primary syphilis	4/4 (100%)	0/4 (0%)
Secondary syphilis	6/6 (100%)	0/6 (0%)
Symptomatic neurosyphilis	7/8 (87.5%)	1/8 (12.5%)
Asymptomatic neurosyphilis	16/16 (100%)	0/16 (0%)
Symptomatic neurosyphilis without our criteria	1/1 (100%)	0/1 (0%)
Latent syphilis	86/92 (83.5%)	6/92 (6.5%)
Treated syphilis	58/61 (95.1%)	3/61 (4.9%)
Without syphilis—other neurological diseases than neurosyphilis	11/126 (8.7%)	115/126 (91.3%)

^a+ve, positive.

^b–ve, negative.

TABLE 2. Results Obtained in Sera and CSF Samples From Individuals With Other Neurological Diseases

Serologic tests in sera	Cell count	Proteins	VDRL	RPR	FTA-Abs	Number
–ve	Normal	Normal	–ve	–ve	–ve	48
–ve	Normal	High	+ve	+ve	–ve	1
–ve	Normal	High	–ve	–ve	–ve	33
–ve	High	High	–ve	–ve	–ve	44

+ve, positive; –ve, negative; CSF, cerebrospinal fluid; VDRL, Venereal Disease Research Laboratory; RPR, Rapid Plasma Reagin; FTA-Abs, Fluorescent Treponemal Antibody Absorption Assay.

TABLE 3. Cell Count, Proteins, and Serological Tests in CSF Samples From Individuals With Primary and Secondary Syphilis

	Cell count	Proteins	VDRL	RPR	FTA-Abs	Number
Primary syphilis (4)	Normal	Normal	–ve	–ve	–ve	4
Secondary syphilis (6)	High	High	–ve	–ve	–ve	1
	High	Normal	–ve	–ve	–ve	1
	Normal	High	–ve	–ve	–ve	1
	Normal	Normal	–ve	–ve	–ve	3

+ve, positive; –ve, negative; CSF, cerebrospinal fluid; VDRL, Venereal Disease Research Laboratory; RPR, Rapid Plasma Reagin; FTA-Abs, Fluorescent Treponemal Antibody Absorption Assay.

TABLE 4. Cell Count, Proteins, and Serological Tests in CSF Samples From Patients With Neurosyphilis

	Cell count	Proteins	VDRL	RPR	FTA-Abs	Number
Symptomatic neurosyphilis (8)	High	High	+ve	+ve	+ve	7
	High	High	–ve	+ve	+ve	1
Symptomatic neurosyphilis without our criteria	High	Normal	–ve	+ve	+ve	1
Asymptomatic neurosyphilis (16)	High	High	+ve	+ve	+ve	9
	High	High	+ve	–ve	+ve	1
	High	High	–ve	–ve	+ve	4
	High	High	–ve	+ve	+ve	2

+ve, positive; –ve, negative; CSF, cerebrospinal fluid; VDRL, Venereal Disease Research Laboratory; RPR, Rapid Plasma Reagin; FTA-Abs, Fluorescent Treponemal Antibody Absorption Assay.

TABLE 5. Cell Count, Proteins, and Serological Tests in CSF Samples From 92 Patients With Latent Syphilis

Cell count	Proteins	VDRL	RPR	FTA-Abs	Number
Normal	Normal	–ve	–ve	–ve	69
Normal	Normal	–ve	–ve	+ve	7
Normal	Normal	+ve	+ve	+ve	1
Normal	High	–ve	–ve	+ve	3
High	High	–ve	–ve	Indeterminate	1
High	High	–ve	–ve	–ve	7
High	Normal	–ve	–ve	+ve	3
High	Normal	–ve	–ve	–ve	1

+ve, positive; –ve, negative; CSF, cerebrospinal fluid; VDRL, Venereal Disease Research Laboratory; RPR, Rapid Plasma Reagin; FTA-Abs, Fluorescent Treponemal Antibody Absorption Assay.

VDRL and RPR. Eight of the reminder 12 CSF samples had raised cell count with high proteins and nonreactive serological tests, with the exception of one, in which the FTA-Abs was indeterminate. From the other four, three

TABLE 6. Cell Count, Proteins, and Serological Tests in CSF Samples From 61 Individuals With Previously Treated Syphilis

Cell count	Proteins	VDRL	RPR	FTA-Abs	Number
Normal	Normal	–ve	–ve	–ve	54
Normal	Normal	–ve	–ve	+ve	6
Normal	Normal	–ve	–ve	Indeterminate	1

+ve, positive; –ve, negative; CSF, cerebrospinal fluid; VDRL, Venereal Disease Research Laboratory; RPR, Rapid Plasma Reagin; FTA-Abs, Fluorescent Treponemal Antibody Absorption Assay.

had a reactive FTA-Abs and a high cell count, whereas in one only cell count was increased.

The results obtained in the CSF samples from 61 patients with previously treated syphilis are discriminated in Table 6.

All of them had normal cell count and proteins. In relation to serological tests, the FTA-Abs was the only reactive test in six and indeterminate in another one.

TABLE 7. Specificity and Sensitivity of VDRL and RPR

	Specificity	Sensitivity
VDRL	286/289 (99%)	17/24 (70.8%)
RPR	287/289 (99.3%)	18/24 (75%)

VDRL, Venereal Disease Research Laboratory; RPR, Rapid Plasma Reagin.

The specificity and the sensitivity of the VDRL and the RPR for current neurosyphilis were determinate taking into account our criteria of neurosyphilis (Table 7). The specificity was 99% for the VDRL test and 99.3% for the RPR, whereas the sensitivity was 70.8 and 75%, respectively, for the VDRL and the RPR.

DISCUSSION

The diagnosis of syphilis is most of the times based on clinical grounds and reactivity of serological tests. Any of the nontreponemal tests (VDRL or RPR) may be used, as they are both similar in relation to sensitivity and specificity (10). Although only the VDRL has been recommended for the diagnosis of neurosyphilis (5,11), to our knowledge only one study comparing the two tests has been performed, in which the RPR was considered to have a low specificity (1).

In this study, we examined 126 CSF samples from patients with other neurological disorders than neurosyphilis. None of the patients in this group had reactivity of the serological tests for syphilis in the CSF, including the RPR, with the exception of one specimen from a patient with hydrocephaly, in which proteins were increased and both the RPR and the VDRL were reactive. These results are not in accordance with Larsen et al. (1), as 14% of their patients with other neurological diseases than neurosyphilis presented a false-positive RPR result. An explanation for the disagreement between the two studies could be the better performance of the technique that is presently available, in relation to that used 20 years ago.

All cases of secondary syphilis studied by us did not present reactivity in any serological test performed in the CSF, although three of them had abnormal cell count and/or high proteins. This may be a consequence of a possible neurological reaction in secondary syphilis, as previously stated by other authors (12), or to be in relation with the HIV infection present in these patients.

Some discrepancies were observed between the RPR and the VDRL in patients with symptoms of neurosyphilis: the RPR was reactive in two patients, one presenting all other criteria used by us for the diagnosis of neurosyphilis and the other with all of these criteria, with the exception of high proteins. In our point of view,

this last patient could be a true neurosyphilis case, in which the VDRL did not react, because he had clinical signs and symptoms of neurosyphilis and most of the parameters were altered. In the asymptomatic neurosyphilis group, the RPR was reactive in two CSF samples from patients with a nonreactive VDRL and the inverse occurred in one. The VDRL and the RPR tests did not identify four patients that fulfilled our criteria of asymptomatic neurosyphilis. This is in agreement with other authors (13–17), who have stated that a nonreactive VDRL does not exclude the diagnosis of neurosyphilis, the same being probably true for the RPR test.

In latent syphilis, all samples were nonreactive in both the VDRL and RPR tests, with the exception of one that also presented a reactive FTA-Abs test. This could be a true case of neurosyphilis, in which proteins and cell count were normal, as serological test titers decreased on follow-up after treatment of the patient. However, it seems that in the latent stage the FTA-Abs may lack specificity, as it was reactive alone in seven CSF samples and together with high cell count in three.

The FTA-Abs was the only reactive test in six CSF samples from patients with treated syphilis, which also confirms that this test may give false-positive results (18,19) when used to diagnose neurosyphilis.

As most patients in this study were infected with HIV, this could originate some false-positive results. However, to our knowledge this has only been described for blood (20,21) and even though no consensus has been found (5,22). Taking that into account and the fact that we are comparing the same type of tests (nontreponemal), we think that our conclusions were not affected by the higher number of HIV patients included in the study.

In view of the results presented here, it seems to us that the RPR is as sensitive and as specific as the VDRL when used in CSF samples. Therefore, it can be concluded that there is no reason for the RPR test not to be used in the diagnosis of neurosyphilis.

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