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Cerebrospinal Fluid *Treponema pallidum* Particle Agglutination Assay for Neurosyphilis Diagnosis

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ABSTRACT Limited data suggest that the cerebrospinal fluid *Treponema pallidum* particle agglutination assay (CSF-TPPA) is sensitive and a CSF *Treponema pallidum* hemagglutination assay (CSF-TPHA) titer of ≥1:640 is specific for neurosyphilis diagnosis. CSF-TPPA reactivity and titer were determined for a convenience sample of 191 CSF samples from individuals enrolled in a study of CSF abnormalities in syphilis (training data set). The sensitivity of a reactive test and the specificity for reactivity at serial higher CSF dilutions were determined. Subsequently, CSF-TPPA reactivity at a 1:640 dilution was determined for all available samples from study participants enrolled after the last training sample was collected (validation data set, $n = 380$). Neurosyphilis was defined as (i) a reactive CSF Venereal Disease Research Laboratory test (CSF-VDRL), (ii) detection of *T. pallidum* in CSF by reverse transcriptase PCR, or (iii) new vision loss or hearing loss. In the training data set, the diagnostic sensitivities of a reactive CSF fluorescent treponemal antibody absorption test (CSF-FTA-ABS) and a reactive CSF-TPPA did not differ significantly (67 to 98% versus 76 to 95%). The specificity of a CSF-TPPA titer of ≥1:640 was significantly higher than that of lower dilutions and was not significantly different from that of CSF-VDRL. In the validation data set, the diagnostic specificity of a CSF-TPPA titer of ≥1:640 was high and did not differ significantly from that of CSF-VDRL (93 to 94% versus 90 to 91%). Ten CSF samples with a nonreactive CSF-VDRL had a CSF-TPPA titer of ≥1:640. If a CSF-TPPA titer of ≥1:640 was used in addition to a reactive CSF-VDRL, the number of neurosyphilis diagnoses would have increased from 47 to 57 (21.3%). A CSF-TPPA titer cutoff of ≥1:640 may be useful in identifying patients with neurosyphilis when CSF-VDRL is nonreactive.

KEYWORDS TPPA, diagnosis, human immunodeficiency virus, neurosyphilis, syphilis

There is no single sensitive and specific test for the diagnosis of neurosyphilis. The cerebrospinal fluid Venereal Disease Research Laboratory test (CSF-VDRL) is considered the "gold standard" test. While CSF-VDRL is specific, its sensitivity is 30 to 70% (1, 2). Conversely, CSF treponemal tests, in particular, the CSF fluorescent treponemal antibody absorption test (CSF-FTA-ABS), are more sensitive than CSF-VDRL, but they lack specificity (3). The sensitivities of CSF treponemal tests are greater than 90% when neurosyphilis is defined as a reactive CSF-VDRL and are lower when the diagnosis of neurosyphilis is based on clinical findings (3). Limited data on the use of the CSF *Treponema pallidum* particle agglutination assay (CSF-TPPA) for neurosyphilis diagnosis suggest that, like CSF-FTA-ABS, it may be diagnostically sensitive (4, 5). The *Treponema pallidum* hemagglutination assay (TPHA) is similar to TPPA, and two studies have suggested that a CSF-TPHA titer of ≥1:640 is specific for neurosyphilis diagnosis (6, 7). CSF-FTA-ABS requires a fluorescence microscope, while TPHA and TPPA are simpler flocculation tests whose results can be read by eye. The purpose of this study was to

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TABLE 1 Characteristics of study participants[a]

Characteristic	Values for: Training data set (n = 191)	Validation data set (n = 380)	P value
No. (%) of male participants	185 (96.9)	373 (98.2)	NS
No. (%) of HIV-infected participants	158 (82.7)	297 (78.2)	NS
Median (IQR) age (yr)	37 (32–43)	41 (32–48)	0.001
Median (IQR) 1/serum RPR titer	64 (32–256)	64 (16–128)	NS
No. (%) of participants:			
With early syphilis[b]	133 (69.6)	284 (74.7)	NS
Treated for uncomplicated syphilis before lumbar puncture	69 (36.1)	203 (53.4)	<0.001
With reactive CSF-VDRL	60 (31.4)	47 (12.4)	<0.001
In whom *T. pallidum* was detected in CSF by RT-PCR	45 (23.6)	41 (10.8)	<0.001
Median (IQR) no. of:			
CSF WBCs	9 (2–33)	5 (2–14)	0.02
CSF RBCs	1 (0–9)	1 (0–14)	NS
No. (%) of participants with new vision or hearing loss	40 (22.0)[c]	95 (25.0)	NS
No. (%) of participants treated for neurosyphilis	105 (55.0)	120 (31.6)	<0.001

[a]CSF, cerebrospinal fluid; RBCs, red blood cells; RPR, rapid plasma reagin; RT-PCR, reverse transcriptase PCR; WBCs, white blood cells; IQR, interquartile range; CSF-VDRL, CSF Venereal Disease Research Laboratory test; NS, not significant.
[b]Early syphilis includes the primary, secondary, and early latent stages.
[c]Data are for 182 patients.

examine the sensitivity of CSF-TPPA for the diagnosis of laboratory and clinically defined neurosyphilis compared to that of CSF-FTA-ABS and to determine whether, like CSF-TPHA, a higher-titer cutoff value of the CSF-TPPA could be used to optimize the diagnostic specificity of the test. In addition, we aimed to compare the performance of CSF-TPPA in HIV-infected and -uninfected individuals with syphilis and to determine the impact of CSF red blood cell (RBC) contamination on diagnostic performance.

RESULTS

Participant characteristics. The characteristics of the individuals included in the training and validation data sets are shown in Table 1. The training data set group was younger than the validation data set group. More individuals in the training data set had CSF abnormalities consistent with neurosyphilis, and more individuals in that group than in the validation data set group were treated for neurosyphilis. This difference was expected because the training data set was a convenience sample that included more participants with a reactive CSF-VDRL. About a third of the individuals in the training data set and about half of the individuals in the validation data set were treated for uncomplicated syphilis before undergoing lumbar puncture (LP) (Table 1). The proportion of individuals treated between 1 and 14 days before LP was 66.7% in the training data set and 75.9% in the validation data set, with the remainder being treated between 15 and 90 days before LP.

The three definitions of neurosyphilis (a reactive CSF-VDRL, detection of *T. pallidum* in CSF by reverse transcriptase PCR [RT-PCR], or new vision or hearing loss) were not mutually exclusive. In the training data set, of 60 individuals with a reactive CSF-VDRL, this was the only definition met in 23 (38.3%); of the 45 with RT-PCR detection of *T. pallidum* in CSF, this was the only definition met in 18 (40.0%); and of the 40 with vision or hearing loss, this was the only definition met in 10 (25.0%). In the validation data set, these proportions were 16 (34.0%) of 47 with reactive CSF-VDRL, 14 (34.2%) of 41 with RT-PCR detection of *T. pallidum* in CSF, and 66 (69.5%) of 95 with vision or hearing loss. In the training data set, 27 (46.6%) individuals with a reactive CSF-VDRL had new vision or hearing loss; in the validation data set, this proportion was 19 (40.4%) of 47.

Training analysis. In the training data set, 93 (48.7%) samples were CSF-FTA-ABS reactive and 102 (53.4%) were CSF-TPPA reactive. There was no significant relationship between the reactivity of CSF-FTA-ABS or the reactivity of CSF-TPPA and previous

TABLE 2 Sensitivity of CSF-FTA-ABS and CSF-TPPA for neurosyphilis diagnosis in the training data set[a]

Test	% sensitivity (95% CI) for the following neurosyphilis definition:		
	Reactive CSF-VDRL	Detection of *T. pallidum* in CSF	New vision or hearing loss
CSF-FTA-ABS	98.3 (95.0–100.0)	66.7 (52.9–80.4)	77.5 (64.6–90.4)
CSF-TPPA	95.0 (89.5–100.0)	75.6 (63.0–88.1)	77.5 (64.6–90.4)
CSF-VDRL		48.9 (34.3–63.5)	67.5 (53.0–82.0)

[a]CI, confidence interval; CSF, cerebrospinal fluid; CSF-FTA-ABS, CSF fluorescent treponemal antibody absorption test; CSF-TPPA, CSF *Treponema pallidum* particle agglutination assay; CSF-VDRL, CSF Venereal Disease Research Laboratory test. CSF-FTA-ABS results graded ≥2+ were considered reactive. CSF-TPPA was considered reactive if the titer was ≥1:80, the initial dilution tested.

treatment for uncomplicated syphilis. We examined the sensitivities of CSF-FTA-ABS and CSF-TPPA for the laboratory and clinical diagnoses of neurosyphilis and found that they did not differ significantly (Table 2). As has been reported previously, the sensitivities of both CSF treponemal tests were the highest when the definition of neurosyphilis was a reactive CSF-VDRL and were lower when the diagnosis was based on clinical abnormalities (3).

Because previous studies had suggested that a CSF-TPHA titer of ≥1:640 was specific for the diagnosis of neurosyphilis, we then examined the specificity of CSF-TPPA for neurosyphilis diagnosis at three successively greater dilutions: 1:160 (number positive = 82 [42.9%]), 1:320 (number positive = 57 [29.8%]), and 1:640 (number positive = 38 [19.9%]) (Table 3). The specificity of each successive increase in dilution of the CSF-TPPA significantly improved when neurosyphilis was defined as a reactive CSF-VDRL or as new vision or hearing loss (1:160 versus 1:320, $P = 0.03$ for both; 1:320 versus 1:640, $P = 0.04$ for both) and showed a trend toward significance when neurosyphilis was defined as detection of *T. pallidum* in CSF (1:160 versus 1:320, $P = 0.06$; 1:320 versus 1:640, $P = 0.09$). The specificities of the 1:320 and 1:640 TPPA dilution cutoffs were not significantly different from the specificities of CSF-VDRL when neurosyphilis was defined as detection of *T. pallidum* in CSF or as new vision or hearing loss. Only the specificities at the lowest CSF-TPPA dilution (1:160) were significantly different from the specificities of CSF-VDRL ($P = 0.04$ for neurosyphilis defined as detection of *T. pallidum* in CSF, $P = 0.006$ for neurosyphilis defined as new vision or hearing loss). Based on these results, we elected to examine the diagnostic specificity of a CSF-TPPA cutoff of reactivity at a 1:640 dilution in the validation data set.

Validation analysis. In the validation data set, 28 (7.4%) of 380 samples had a CSF-TPPA titer of ≥1:640. The diagnostic specificity of a positive CSF-TPPA result at a dilution of ≥1:640 was high for all three neurosyphilis definitions (Table 4) and did not differ significantly from that of CSF-VDRL for neurosyphilis defined as detection of *T. pallidum* in CSF or new vision or hearing loss. Ten CSF samples with a nonreactive CSF-VDRL had a reactive CSF-TPPA titer of ≥1:640. If a CSF-TPPA titer of ≥1:640 was used in addition to a reactive CSF-VDRL for neurosyphilis diagnosis, the number of

TABLE 3 Specificity of CSF-TPPA cutoffs for neurosyphilis diagnosis in the training data set[a]

Test	% specificity (95% CI) for the following neurosyphilis definition:		
	Reactive CSF-VDRL	Detection of *T. pallidum* in CSF	New vision or hearing loss
CSF-TPPA titer of:			
≥1:160	75.6 (68.2–83.0)	63.0 (55.2–70.8)	63.4 (55.5–71.3)
≥1:320	86.3 (80.4–92.2)	73.3 (66.1–80.5)	75.4 (68.3–82.5)
≥1:640	93.9 (89.8–98.0)	81.5 (75.2–87.8)	85.2 (79.4–91.0)
CSF-VDRL		74.0 (66.9–81.1)	78.2 (71.4–85.0)

[a]CI, confidence interval; CSF, cerebrospinal fluid; CSF-TPPA, CSF *Treponema pallidum* particle agglutination assay; CSF-VDRL, CSF Venereal Disease Research Laboratory test.

TABLE 4 Specificity of CSF-TPPA titer of ≥1:640 and CSF-VDRL for neurosyphilis diagnosis in the validation data set[a]

Test	% specificity (95% CI) for the following neurosyphilis definition:		
	Reactive CSF-VDRL	Detection of *T. pallidum* in CSF	New vision or hearing loss
CSF-TPPA titer of ≥1:640	97.0 (95.2–98.8)	93.8 (91.2–96.4)	93.3 (90.4–96.2)
CSF-VDRL		91.2 (88.1–94.2)	90.2 (86.7–93.6)

[a]CI, confidence interval; CSF, cerebrospinal fluid; CSF-TPPA, CSF *Treponema pallidum* particle agglutination assay; CSF-VDRL, CSF Venereal Disease Research Laboratory test.

participants with neurosyphilis would have increased from 47 to 57 (21.3%). The specificity of the CSF-TPPA titer of ≥1:640 did not differ significantly between HIV-infected and -uninfected individuals using any of the three neurosyphilis definitions (data not shown). There was no significant relationship between the CSF red blood cell (RBC) concentration and the reactivity of CSF-TPPA or a CSF-TPPA titer of ≥1:640 (data not shown). However, only 7 of 380 (1.8%) samples had more than 500 RBCs/μl.

DISCUSSION

CSF-VDRL is considered the "gold standard" test for neurosyphilis diagnosis. Reports of its sensitivity and specificity vary depending on how neurosyphilis is defined, but the preponderance of data suggests that the test lacks sensitivity. In their extensive monograph on neurosyphilis published in 1946, when the disease was common, Merritt and colleagues documented nonreactive CSF Wassermann tests, the predecessor of CSF-VDRL, in 8 to 19% of patients with early neurosyphilis, including meningitis and meningovasculitis (8), the symptomatic forms that are the most common today. In our analysis, the sensitivity of CSF-VDRL ranged from 49 to 68%, using laboratory and clinical diagnostic criteria. CSF-FTA-ABS has a greater sensitivity than CSF-VDRL (3), but its use requires a fluorescence microscope, and the results are not easily quantified. In contrast to CSF-FTA-ABS, CSF-TPPA does not require specialized equipment and determination of a titer is straightforward. In a few studies, CSF-TPPA has been shown to be highly sensitive (4, 5), and the use of a cutoff value for CSF-TPHA, a test similar to CSF-TPPA, increases the specificity for neurosyphilis diagnosis (6, 7).

In this study, we investigated the diagnostic performance of CSF-TPPA. Starting with a training data set, we determined that the diagnostic sensitivity of CSF-TPPA did not differ significantly from that of CSF-FTA-ABS, and we determined that a CSF-TPPA titer cutoff of ≥1:640 had a high diagnostic specificity for all three definitions of neurosyphilis. In a subsequent validation data set, we confirmed the high specificity of the CSF-TPPA cutoff and found that it did not differ between HIV-infected and -uninfected patients with syphilis. Treponemal IgG antibodies likely passively diffuse into CSF, which explains why conventional CSF treponemal test reactivity lacks diagnostic specificity (9). The improvement in specificity obtained using a high-titer cutoff suggests that there is a CSF treponemal antibody concentration above which intrathecal production is more likely than passive diffusion.

Limitations of our study should be noted. Our participants were referred because of a perceived increased risk of neurosyphilis and, as such, do not represent all patients with syphilis. On the other hand, in clinical practice, testing for neurosyphilis is considered only for patients who merit diagnostic suspicion. We used three definitions of neurosyphilis, two laboratory and one clinical, that were not mutually exclusive. These definitions, at least for reactive CSF-VDRL and new vision or hearing loss, correspond to the definitions of neurosyphilis used in clinical practice. Most of our study participants were HIV-infected men who have sex with men, reflecting the demographics of syphilis in our community. However, we did not identify a difference in the specificity of a CSF-TPPA titer cutoff of ≥1:640 between HIV-infected and HIV-uninfected study participants. Our work differs from previous studies of CSF-TPPA

or CSF-TPHA because of its prospective nature with standardized assessments and because of its relatively large sample size (4–7).

Neurosyphilis remains a difficult diagnosis to establish and exclude. Our results suggest that a reactive CSF-TPPA at a titer of ≥1:640 may be useful in identifying patients with neurosyphilis when CSF-VDRL is nonreactive. Future studies are required to confirm our findings. A longitudinal study of individuals whose neurosyphilis diagnosis is based upon CSF-TPPA will be particularly informative.

MATERIALS AND METHODS

Study participants. Participants were enrolled in a prospective study of CSF abnormalities in syphilis conducted in Seattle, WA, from 18 December 2000 to 6 June 2014 (10). Study eligibility included clinical or serological evidence of syphilis and an assessment by the referring provider that the patient was at risk for neurosyphilis. Reasons for referral to the study included, but were not restricted to, (i) neurological findings, particularly new vision or hearing loss; (ii) a serum rapid plasma reagin (RPR) titer of ≥1:32; and (iii) in HIV-infected individuals, a peripheral blood $CD4^+$ T cell count of ≤350/μl. The last two criteria for risk for neurosyphilis are supported by published data (10–12). At study entry, participants underwent a structured history and neurological examination that included assessment of vision and hearing, LP, and venipuncture. The results reported here are from the study entry visit. The study protocol was reviewed and approved by the University of Washington Institutional Review Board, and human experimentation guidelines were followed in the conduct of this research. Written informed consent was obtained from all participants.

Laboratory methods. Determination of the plasma HIV RNA concentration, CSF-VDRL, and enumeration of CSF RBCs and white blood cells (WBCs) were performed in a Clinical Laboratory Improvement Amendments (CLIA)-approved hospital clinical laboratory. Serum RPR tests were performed in a research laboratory using published methods (13). Identification of *T. pallidum* 16S rRNA in CSF was performed using RT-PCR as previously described (10). CSF-FTA-ABS and CSF-TPPA were performed in a research laboratory according to the manufacturers' methods for serum but using cell-free CSF instead. CSF-FTA-ABS results graded as ≥2+ were considered reactive, and CSF-TPPA was considered reactive if the titer was ≥1:80, the initial dilution tested. CSF-FTA-ABS reactivity, CSF-TPPA reactivity, and the CSF-TPPA titer (for CSF-TPPA-reactive samples) were determined for a convenience sample of 191 participants selected to increase the proportion with a reactive CSF-VDRL (training data set). To confirm our findings in the training data set, we subsequently determined CSF-TPPA reactivity for all available samples from study participants enrolled after the last training sample was collected ($n = 380$, validation data set). In the validation data set, CSF-TPPA reactivity was tested at a 1:320 dilution for samples that were CSF-TPPA reactive at 1:80, and CSF-TPPA reactivity was tested at a 1:640 dilution for those that were reactive at 1:320. The individuals who determined CSF-FTA-ABS and CSF-TPPA reactivity were blind to whether the study participants met one or more definitions of neurosyphilis. CSF samples were collected before neurosyphilis treatment, but some study participants were treated for uncomplicated syphilis before collection of CSF (Table 1).

Statistical methods. Neurosyphilis was defined according to laboratory criteria (i) as a reactive CSF-VDRL; (ii) as detection of *T. pallidum* in CSF by RT-PCR, regardless of the clinical findings; and (iii) by clinical criteria as new vision loss or hearing loss, regardless of CSF-VDRL reactivity or detection of *T. pallidum* in CSF. The rationale for these definitions includes the fact that they are commonly used in clinical care (1). The CSF-VDRL is considered the gold standard for neurosyphilis diagnosis, despite its limited sensitivity (1). Vision and hearing loss were the most common clinical findings in individuals with symptomatic neurosyphilis in our study. Detection of *T. pallidum* in CSF is not routinely available in clinical care but afforded us a different laboratory neurosyphilis definition that could be compared to CSF-VDRL reactivity.

Descriptive statistics are reported as number (percent) or median (interquartile range [IQR]). Associations between categorical variables were assessed by the chi-square test or Fisher's exact test, associations between continuous and categorical variables were determined by the Mann-Whitney U test, and associations between continuous variables were assessed by use of the Spearman rank correlation coefficient using SPSS (version 19) software. Sensitivity and specificity were calculated using standard formulae. Differences in sensitivity and specificity were compared using the two-sample test of proportions with Stata (version 11.2) software. All tests were two-tailed. P values of <0.05 were considered statistically significant.

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We have no conflicts of interest relevant to this work.

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