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Clinical Prediction and Diagnosis of Neurosyphilis in HIV-Infected Patients with Early Syphilis

Jeannot Dumaresq,[a] Stéphanie Langevin,[b] Simon Gagnon,[b] Bouchra Serhir,[c] Benoît Deligne,[d] Cécile Tremblay,[b,c] Raymond S. W. Tsang,[e] Claude Fortin,[b,f] François Coutlée,[b,f] Michel Roger[b,f,g]

Département de Microbiologie et d'Infectiologie, Centre Hospitalier Affilié Universitaire Hôtel-Dieu de Lévis, Lévis, Québec, Canada[a]; Département de Microbiologie et d'Infectiologie, Centre Hospitalier de l'Université de Montréal, Montréal, Québec, Canada[b]; Laboratoire de Santé Publique du Québec, Sainte-Anne-de-Bellevue, Québec, Canada[c]; Département de Médecine Interne et Vasculaire, Centre Hospitalier de l'Université de Montréal, Montréal, Québec, Canada[d]; Division of Syphilis Diagnostics and Vaccine Preventable Bacterial Diseases, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada[e]; Département de Microbiologie et d'Immunologie de l'Université de Montréal, Montréal, Québec, Canada[f]; Laboratoire d'Immunogénétique, Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montréal, Québec, Canada[g]

The diagnosis of neurosyphilis (NS) is a challenge, especially in HIV-infected patients, and the criteria for deciding when to perform a lumbar puncture (LP) in HIV-infected patients with syphilis are controversial. We retrospectively reviewed demographic, clinical, and laboratory data from 122 cases of HIV-infected patients with documented early syphilis who underwent an LP to rule out NS, and we evaluated 3 laboratory-developed validated real-time PCR assays, the *Treponema pallidum* particle agglutination (TPPA) assay, the fluorescent treponemal antibody absorption (FTA-ABS) assay, and the line immunoassay INNO-LIA Syphilis, for the diagnosis of NS from cerebrospinal fluid (CSF) samples of these patients. NS was defined by a reactive CSF-VDRL test result and/or a CSF white blood cell (WBC) count of >20 cells/μl. Thirty of the 122 patients (24.6%) had early NS. Headache, visual symptoms, a CD4 cell count of <500 cells/μl, and viremia, as defined by an HIV-1 RNA count of ≥50 copies/ml, were associated with NS in multivariate analysis ($P = <0.001$ for each factor). Blood serum rapid plasma reagin (RPR) titers were not associated with early NS ($P = 0.575$). For the diagnosis of NS, the PCR, FTA-ABS, TPPA, and INNO-LIA assays had sensitivities of 58%, 100%, 68%, and 100%, specificities of 67%, 12%, 49%, and 13%, and negative predictive values of 85%, 100%, 84%, and 100%, respectively. Visual disturbances, headache, uncontrolled HIV-1 viremia, and a CD4 cell count of <500 cells/μl were predictors of NS in HIV-infected patients with early syphilis, while blood serum RPR titers were not; therefore, RPR titers should not be used as the sole criterion for deciding whether to perform an LP in early syphilis. When applied to CSF samples, the INNO-LIA Syphilis assay easily helped rule out NS.

Neurosyphilis (NS), which is the involvement of the central nervous system by *Treponema pallidum* subsp. *pallidum*, is one of the most feared complications of syphilis. For epidemiological and possibly immunological reasons (1, 2), NS is now more commonly seen in patients infected with HIV than it was previously (3–5), and a significant proportion of these cases occur during the early phase of infection (6). The criteria for deciding when to perform a lumbar puncture (LP) in HIV-infected patients with syphilis are controversial. While the latest guidelines from the Centers for Disease Control and Prevention (CDC) (7) recommend cerebrospinal fluid (CSF) examination only if a patient has either neurological or ophthalmic symptoms or signs, evidence of active tertiary syphilis, or serologic treatment failure, whether or not they are infected with HIV, other guidelines still propose performing an LP in HIV-infected patients with rapid plasma reagin (RPR) titers of ≥1:32 or a CD4 cell count of <350 cells/μl (8, 9). Some experts even recommend cerebrospinal fluid (CSF) examination of all HIV-infected patients with syphilis (8, 10).

Neurosyphilis diagnosis has always been a challenge due to the lack of a gold standard. A reactive CSF-VDRL test is considered to be highly specific and thus establishes the diagnosis of NS in a patient with a reactive serum treponemal test. However, since its sensitivity ranges from as low as 22% up to 69% (11), a nonreactive test cannot exclude the diagnosis of NS, and in those cases, one has to rely on the presence or absence of CSF pleocytosis or elevated protein concentrations for a presumptive diagnosis. However, both of these criteria suffer from poor specificity, particularly in cases of HIV coinfection (12–15). In this setting, a pleocytosis threshold of >20 leukocytes/μl is commonly used instead of the traditional >5 leukocytes/μl threshold used to suspect the presence of NS. Tests that are specific for treponemal antibodies have also been studied and used on CSF samples, including the fluorescent treponemal antibody-absorption (FTA-ABS) test and the *Treponema pallidum* particle agglutination (TPPA) test. While these tests are generally considered to have a high negative predictive value (NPV) for NS (7), a recent review pointed out that their NPVs vary from as low as 58.3% to as high as 100% with subjects having serological evidence of syphilis but without NS as comparators (16). Importantly, most of these studies did not include HIV-infected subjects. Detecting live *T. pallidum* in the CSF is possible using rabbit infectivity testing, but this cumbersome method requires live virulent bacteria, and even if it can detect as few as one to 10 organisms, its sensitivity and specificity for the diagnosis of NS are imperfect (17). Several PCR tests targeting *T.*

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Address correspondence to Jeannot Dumaresq, jeannot_dumaresq@ssss.gouv.qc.ca.

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pallidum have been developed (18); their sensitivities with CSF samples have generally been poor (19), but most of these tests were not based on real-time PCR methods, which may be more sensitive than conventional endpoint PCR methods. Moreover, none of these studies focused on the diagnosis of NS in HIV-infected patients.

Because the criteria for CSF examination based on blood serum RPR titers and CD4 cell counts were derived from cohort studies that recruited patients at all stages of the disease (early and late syphilis), our first objective was to study the predictive factors of NS in a cohort of HIV-infected patients who were exclusively at the early stage of syphilis. Our second objective was to evaluate a real-time PCR method and three different tests that are specific for treponemal antibodies (fluorescent treponemal antibody absorption [FTA-ABS] test, *T. pallidum* particle agglutination [TPPA] test, and the line immunoassay INNO-LIA Syphilis) for the diagnosis of NS in an HIV-infected population with early syphilis.

MATERIALS AND METHODS

Population and clinical specimens. We retrospectively reviewed all cases of HIV-infected patients with documented early syphilis who were referred from September 2006 to June 2009 to the Centre Hospitalier de l'Université de Montréal (CHUM) and underwent an LP to rule out NS according to the Canadian Guidelines on Sexually Transmitted Infections (8), either because they had a blood serum RPR titer of ≥1:32, neurological and/or ophthalmic symptoms or signs, or a CD4 cell count of <350 cells/μl. Early syphilis was defined as (i) a reactive blood serum enzyme immunoassay (EIA) result for syphilis and positive RPR test confirmed by a reactive blood serum TPPA and a negative blood serum EIA result during the past year for patients without a history of syphilis or (ii) a documented increase in the blood serum RPR titer by more than 2 dilutions during the past year in patients with a history of syphilis. Neurosyphilis was defined by a reactive CSF-VDRL test result and/or a CSF white blood cell (WBC) count of >20 cells/μl. The cases were subdivided into confirmed NS, defined by a reactive CSF-VDRL test result, and presumptive NS, defined by a CSF WBC count of >20 cells/μl with a nonreactive CSF-VDRL test result. Patients with missing data, syphilis of unknown duration, or a history of NS or those who were treated with penicillin prior to LP were excluded from our study. We were left with 122 patients after these exclusions. Fifty routine CSF samples were used for the evaluation of the analytical specificities of real-time PCR assays.

The following demographic and clinical data were collected from existing patient medical records: age, gender, clinical findings frequently associated with early NS (headache, visual symptoms, hypoacusis, tinnitus, cranial nerve abnormalities, and motor and sensory symptoms), stage of syphilis, antiretroviral therapy (ART) status (at the time of lumbar puncture), CD4 cell count and HIV-1 viral load, blood serum RPR titers, CSF WBC count, and VDRL test result. Visual symptoms were reported as diplopia, blurred vision, flashes, or a subjective decrease in vision. Serum RPR titers (Wampole, Inverness Medical, Princeton, NJ, USA) were analyzed using various cutoff values (titers of ≥1:16, ≥1:32, ≥1:64, ≥1:128, and ≥1:256). We analyzed the data for patients using CD4 cell count cutoff values of 500, 350, and 200 cells/μl. HIV viremia was either classified as uncontrolled (≥50 HIV RNA copies/ml) or controlled (<50 HIV RNA copies/ml). The CSF samples were kept frozen at −80°C and were anonymized and retrospectively tested. The study was approved by the CHUM institutional review board.

Treponemal tests. The syphilis EIA was performed with the Captia syphilis TA kit (Trinity Biotech, Bray, Ireland). The FTA-ABS double-staining test (Zeus Scientific, Branchburg, NJ, USA) was performed at the National Microbiology Laboratory in Winnipeg, Manitoba, Canada. In accordance with the manufacturer's instructions, samples repeatedly found to be minimally reactive (1+) by FTA-ABS were considered positive, since every patient had clinical evidence of treponemal infection. The TPPA (Fujirebio, Tokyo, Japan) and INNO-LIA Syphilis (Innogenetics, Zwijnaarde, Belgium) assays were performed at the Laboratoire de Santé Publique du Québec (LSPQ), Canada. The treponemal tests were performed by research scientists who were blinded to the results of the PCR and conventional CSF analyses.

Microorganism strains. Forty-five different microorganisms were used for the evaluation of the PCR analytical specificity. *Borrelia burgdorferi* and *Leptospira* strains were kindly provided by Antonia Dibernardo of the Zoonotic Diseases and Special Pathogens branch of the Public Health Agency of Canada (Winnipeg, Manitoba, Canada). All other mircroorganisms were provided by the CHUM.

Construction of synthetic control DNA templates. Templates were obtained from a rabbit testis infected with *T. pallidum* subsp. *pallidum* strain Nichols, provided by the LSPQ. Primer pairs amplifying whole target genes were designed, and the resulting amplicons were cloned using the TOPO TA Cloning kit (Life Technologies). The positive clones were sequenced and then purified using the QIAprep Spin miniprep kit (Qiagen).

Sample preparation and real-time PCR. DNA was extracted from 400 μl of each CSF sample in a designated biological safety cabinet using the QIAamp DNA minikit (Qiagen, Toronto, Ontario, Canada). The microorganisms were suspended in 200 μl of normal saline, and the nucleic acids were extracted using the NucliSENS easyMAG automated system (bioMérieux, Saint-Laurent, Quebec, Canada).

Three different PCRs were developed, each targeting a different *T. pallidum* gene: *polA*, Tpp47, and *bmp*. Each PCR assay was performed in a final reaction mixture volume of 20 μl, containing 5 μl of purified specimen or synthetic control, 10 μl of LightCycler 480 Probes Master (Roche Diagnostics, Laval, Quebec, Canada), optimized concentrations of each primer and hydrolysis probe (Table 1), and water. Real-time PCR tests were performed on a Rotor-Gene 6000 (Corbett Life Science, Sydney, Australia). Master mixes were prepared before each run in a preamplification area, and the pipettes were cleaned before each PCR assay with RNase AWAY reagent (Life Technologies, Burlington, Ontario, Canada). After the enzyme activation step (95°C for 10 min), the amplification conditions for 50 cycles were 95°C for 10 s and 60°C for 30 s. Fluorescence acquisition was obtained at the end of each cycle. The analytical sensitivity of each PCR assay was determined using serial dilutions of the synthetic controls in the presence of approximately 80 ng of human genomic DNA (Roche Diagnostics, Laval, Quebec, Canada). Samples that were positive for only one target out of 3 (discordant samples) were retested for the two other targets in a subsequent run, and 4 Tpp47 amplification products were sequenced. Inhibition and efficiency of extraction were controlled in every CSF sample using a limiting concentration of an exogenous heterologous template (external control), a plasmid containing the G4 gene from *Arabidopsis thaliana* added before each sample preparation under the conditions described above with optimized primers and probe concentrations (Table 1). Positive and negative controls were included in every PCR run.

Statistical analysis. *P* values were calculated using Fisher's exact test, except for age and CD4 cell counts between participants with and without NS, who were compared using a nonparametric Mann-Whitney test. The association between each of the putative predictors in the univariate analysis and the presence of NS was investigated using multivariate logistic regression to derive odds ratios (OR) and respective 95% confidence intervals (CI) as estimates of the relative risks using the Statistica version 6 software (StatSoft, Tulsa, OK). *P* values of <0.05 were considered statistically significant.

RESULTS

Factors predictive of neurosyphilis. Of 122 patients, 121 were men (99.2%), and all of them were men who have sex with men. Most participants were Caucasian (93.3%). The median age of the participants was 42 years (range, 22 to 66). Two patients (1.6%) had primary syphilis, 60 (49.2%) had secondary syphilis, and 60

TABLE 1 Nucleotide sequences, concentrations of primers and probes, and expected amplicon lengths

Oligonucleotide[a]	Target gene	Sequences (5′ to 3′)[b]	Amplicon length (bp)	Concentration (nM)
TPpolA-F3	*polA*	GATTAACGACAGCGGTGCG	140	300
TPpolA-R3	*polA*	GGTACTGTACGTGCCGAAAGG	140	300
TPpolA-P3	*polA*	6-FAM-ACAGGAGCGTGTGCAAACTCCG-BHQ-1	140	200
TPp47-F2	Tpp47	ACGAAGTGAAACTTCTCCTTTGC	110	300
TPp47-R2	Tpp47	GACAGCGAGGAATACAAGATTACG	110	300
TPp47-P2	Tpp47	6-FAM-AAGTTTGTCCCAGTTGCGGTTCCTC-BHQ-1	110	100
TPbmp-F2	*bmp*	AATAAATTCACTTCGGCGATAGG	92	300
TPbmp-R2	*bmp*	AGATACATAGTTCCCGCTCTGTTG	92	600
TPbmp-P2	*bmp*	6-FAM-CAAACCCCATACCCGCCACG-BHQ-1	92	100
CSG4-F	G4	TGTGGGCAGGGCATACC	58	300
CSG4-R	G4	AGCAATGATCCTCCCAAAGC	58	600
CSG4-TM	G4	6-FAM-CCCACTGTCTTCTATC-BHQ-1	58	200

[a] CSG4, chlorophyll synthase G4 gene from *Arabidopsis thaliana*.
[b] 6-FAM, 6-carboxyfluorescein; BHQ, black hole quencher.

(49.2%) were in the early latent stage. Eighty-five (69.7%) patients were on ART. Thirty of the 122 patients (24.6%) had a diagnosis of NS according to our criteria. Of these, 4 (13.3%) had a reactive CSF-VDRL test result only, 11 (36.7%) had a CSF-WBC count of >20 cells/μl only, and 15 (50.0%) had both. None of the CSF samples had gross blood contamination, and the CSF-VDRL reactive test samples had a median of 3 red blood cells/μl (range, 0 to 68). Only 2 of the 66 asymptomatic patients had a reactive CSF-VDRL test result. Most NS cases were symptomatic (80%), with 47% reporting headache, 47% reporting visual disturbances, and 20% reporting both. Only 14% of symptomatic patients had hypoacusis.

All patients with NS had at least one of the following criteria: a CD4 cell count of <500 cells/μl, detectable HIV-1 viremia, headache, or visual symptoms. Among patients who did not fulfill any of these criteria, no case of NS was found, for a negative predictive value of 100% (95% CI, 81% to 100%). The differences between patients with and without NS on univariate analysis are reported in Table 2. All significant factors that were associated with NS in the univariate analysis, as well as blood serum RPR titers, were then studied in a multivariate model (Table 3). Because of the expected colinearity between ART and viremia control, only the latter was included in the multivariate model.

Performance of PCR. The analytical sensitivities of the real-time PCR assays were 10 copies/reaction for *polA* and between 1 and 10 copies/reaction for Tpp47 and *bmp*, with Tpp47 being the most sensitive (data not shown). The 44 various microorganisms all tested negative with the 3 PCR assays (100% analytical specificity). The 50 CSF control samples all tested negative with the 3 PCR assays (100% clinical specificity). The external control was adequately detected in every CSF sample.

Of the 122 study samples, 108 had enough CSF remaining to be analyzed by PCR. These 108 patients were all men, with a median age of 43 years (range, 22 to 64 years). Twenty-four (22%) had NS, of whom 4 (17%) had a reactive CSF-VDRL test result only, 9 (38%) had a CSF WBC count of >20 cells/μl only, and 11 (46%) had both criteria. The *polA* gene was detected in 11 samples (10%), the Tpp47 gene was detected in 39 samples (36%), and the *bmp* gene was detected in 15 samples (14%). At least one gene was detected in 42 samples (39%). While 17 of these samples (40%) were positive with ≥2 PCR assays, 25 (60%) were positive for only one target, mostly for Tpp47 (23 out of 25). Considering that the presence of *T. pallidum* DNA was confirmed in 11 of these 25 discordant samples by amplifying at least one of the other 2 targets in repeat testing, most PCR-positive samples had <10 copies/reaction (data not shown), and Tpp47 PCR products were successfully sequenced, all the samples in which at least one PCR target was detected were considered positive for *T. pallidum* DNA. The performance characteristics of PCR for the diagnoses of confirmed and presumptive NS are shown in Table 4.

Performance of FTA-ABS, TPPA, and INNO-LIA assays. Of the 108 CSF samples analyzed by PCR, 104 had sufficient volume remaining to be analyzed by both the TPPA and INNO-LIA assays, and 100 had sufficient volume remaining to be analyzed by the FTA-ABS assay. Indeterminate TPPA ($n = 4$) or INNO-LIA ($n = 21$) assay results were excluded. The performance characteristics of the FTA-ABS, TPPA, and INNO-LIA assays for the diagnoses of confirmed and presumptive NS are shown in Table 4, and the performance characteristics of the PCR, FTA-ABS, TPPA, and INNO-LIA assays for the diagnoses of symptomatic and asymptomatic NS are shown in Table 5.

Of the 100 CSF samples that were analyzed by every test (PCR and all treponemal tests), 75 samples gave interpretable results after exclusion of the indeterminate TPPA and INNO-LIA test results. Of these 75 patients, 19 had a diagnosis of NS and 10 (53%) of these cases were reactive by PCR and all treponemal tests. They were all reactive by the INNO-LIA and FTA-ABS tests, but only 11 (59%) and 15 (79%) were reactive by the PCR and TPPA tests, respectively.

DISCUSSION

Our data on HIV-infected patients with early syphilis showed that visual symptoms, headache, a CD4 count of <500 cells/μl, and uncontrolled viremia were significantly associated with NS, whereas blood serum RPR titer was not. A CD4 cell count of <350 cells/μl is a previously recognized risk factor for NS (20); we additionally found an association between a CD4 cell count of <500 cells/μl and NS, which suggests that we should not reserve LPs only for patients with <350 CD4 cells/μl. However, we did not demonstrate any association between NS and a CD4 cell count of <200 cells/μl. We might hypothesize that severely immunocompromised patients are less likely to mount an inflammatory response in the central nervous system (CNS), but it might also be attributed to our small sample size in that category. The exclusion

TABLE 2 Clinical and laboratory characteristics and their association with NS in univariate analysis[a]

Patient and laboratory characteristics	Data by NS status:		*P*	Crude OR (95% CI)[b]
	With NS ($n = 30$)	Without NS ($n = 92$)		
Median age (range) (yr)	39 (22–66)	43 (23–64)	0.034	0.95 (0.90–0.99)
Stage of syphilis (*n* [%])			0.400	
Primary	1 (3)	1 (1)		
Secondary	17 (57)	43 (47)		
Early latent	12 (40)	48 (52)		
ART (*n* [%])[c]	10 (33)	75 (82)	<0.001	0.11 (0.05–0.29)
Viral load ≥ 50 (*n* [%])	21 (70)	24 (26)	<0.001	7.44 (2.88–19.18)
CD4 cell count				
Mean (range)	333 (30–560)	464 (80–1,180)	0.004	0.52 (0.33–0.82)
≤500 (*n* [%])	27 (90)	57 (62)	0.008	5.53 (1.54–19.83)
≤350 (*n* [%])	18 (60)	29 (32)	0.007	3.26 (1.38–7.71)
≤200 (*n* [%])	4 (13)	8 (9)	0.334	1.61 (0.45–5.80)
RPR titers[d]				
Median (range)	256 (32–8,192)	128 (4–8,192)	0.639	
≥1:16 (*n* [%])	30 (100)	86 (94)	0.364	41.86 (0.34–>1,000)
≥1:32 (*n* [%])	30 (100)	82 (89)	0.119	73.17 (0.63–>1,000)
≥1:64 (*n* [%])	28 (93)	75 (82)	0.154	3.17 (0.69–14.63)
≥1:128 (*n* [%])	22 (73)	55 (60)	0.200	1.85 (0.75–4.60)
≥1:256 (*n* [%])	16 (53)	45 (49)	0.834	1.19 (0.52–2.73)
Symptoms (*n* [%])				
Any	24 (80)	32 (35)	<0.001	7.50 (2.75–20.43)
Headache	14 (47)	18 (20)	0.004	3.60 (1.47–8.78)
Visual	14 (47)	10 (11)	<0.001	7.18 (2.69–19.16)
Hypoacusis	5 (14)	11 (12)	0.533	0.82 (0.21–3.15)

[a] NS, neurosyphilis.
[b] OR, odds ratio; CI, confidence interval.
[c] ART, antiretroviral therapy.
[d] RPR, rapid plasma regain.

of patients with a CD4 cell count of <500 cells/μl, detectable HIV-1 viremia, headache, or visual symptoms virtually ruled out all patients with CSF abnormalities that are consistent with NS. Conversely, all patients having CSF abnormalities consistent with NS had at least one of these criteria. These findings might help clinicians limit the number of LPs performed while a maximum number of cases are still identified and treated. Previous reports have shown an association between NS and an RPR titer of >1:32 in HIV-infected patients (20, 21, 37). However, these studies included all stages of syphilis. Our results suggest that in early syphilis, the RPR titer magnitude may not be as predictive of NS as it is during the later stages of disease, and it probably should not be used as the sole criterion for deciding whether to perform an LP.

TABLE 3 Clinical and laboratory characteristics and their association with NS in the multivariate model

Variable	Odds ratio	95% CI[a]	*P*
Age	0.99	0.93–1.05	0.699
Headache	4.68	1.45–15.09	0.009
Visual symptoms	9.65	2.62–35.45	<0.001
CD4 cell count ≤ 500	8.68	1.73–43.37	0.008
Viral load ≥ 50	7.22	2.13–24.55	0.001
RPR[b]	1.00	0.99–1.01	0.575

[a] CI, confidence interval.
[b] RPR, rapid plasma reagin.

Several studies have evaluated PCR for the diagnosis of NS in adults (10, 19, 22–29). However, the results obtained in these studies are difficult to compare to our results for several reasons. Most of these studies used conventional PCR techniques, had different criteria for defining NS, included very few patients, or did not focus exclusively on HIV-infected patients with early syphilis. The sensitivities for the diagnosis of NS in any syphilis stage have generally been low, ranging from 0% to 71%. Some factors might explain why the sensitivity of our PCR assays (58%) for the diagnosis of NS was better than those in most other studies. First, the analytical sensitivities of our assays combined with the nucleic acid extraction method we used possibly made them more efficient. Real-time PCR is usually more sensitive than conventional PCR while being less prone to contamination. Moreover, our test consisted of 3 different assays run in parallel, increasing the chance of detecting *T. pallidum* DNA even when present in very low concentrations. Second, all our patients had early syphilis. *T. pallidum* is thought to invade the central nervous system (CNS) early in the course of infection, but it is less commonly detected from CSF samples obtained in late stages of the disease (10, 17, 30). This phenomenon might also explain the lower specificity of

TABLE 4 Performance characteristics of PCR, FTA-ABS, TPPA, and INNO-LIA assays for diagnosis of confirmed and presumptive neurosyphilis in HIV-infected patients with early syphilis

Assay[a]	NS status[b]	No. of patients	No. of NS cases	No. of test-positive cases	Performance characteristics (%)[d]				Likelihood ratios	
					Sensitivity	Specificity	PPV	NPV	Positive	Negative
PCR	C or P	108	24	14	58	67	33	85	1.75	0.63
	C	108	15	6	40	61	14	86	1.03	0.98
	P	93	9	8	89	67	22	98	2.67	0.17
FTA-ABS	C or P	100	23	23	100	12	25	100	1.13	0
	C	100	15	15	100	11	17	100	1.12	0
	P	85	8	8	100	12	11	100	1.13	0
TPPA[c]	C or P	100	22	15	68	49	27	84	1.33	0.65
	C	100	15	10	67	47	18	89	1.26	0.71
	P	85	7	5	71	49	11	95	1.39	0.59
INNO-LIA[c]	C or P	83	20	20	100	13	27	100	1.15	0
	C	83	12	12	100	11	16	100	1.13	0
	P	71	8	8	100	13	13	100	1.15	0

[a] FTA-ABS, fluorescent treponemal antibody absorption; TPPA, *Treponema pallidum* particle agglutination.
[b] NS, neurosyphilis; C, confirmed (CSF-VDRL test reactive); P, presumptive (CSF WBC of >20 cells/μl only).
[c] Patients with indeterminate results were excluded (TPPA, $n = 4$; INNO-LIA, $n = 21$).
[d] PPV, positive predictive value; NPV, negative predictive value.

our test, since many patients will experience CNS dissemination early during infection but will eventually clear the organisms without necessarily developing an inflammatory reaction. Third, all our patients were infected with HIV, and we noticed a statistically significant association between low CD4 cell count and the detection of *T. pallidum* DNA in CSF samples (data not shown). Other studies have also suggested that HIV-induced immunodeficiency might delay the clearance of CNS organisms (12, 30).

When considering only the presumptive cases of NS (i.e., those with CSF WBC counts of >20 cells/μl but a negative CSF-VDRL test result), a negative CSF *T. pallidum* PCR had a high NPV (98%). We also noticed a statistically significant association between the degree of pleocytosis and the detection of *T. pallidum* DNA in CSF samples (data not shown). All these findings support the use of a higher CSF WBC count threshold for the diagnosis of NS in HIV-infected patients so as to enhance specificity, as has been proposed by some authors (1, 10). It is important to emphasize, however, that the performance characteristics of this higher threshold for the diagnosis of NS have not been established due to the lack of a gold standard. We cannot exclude the possibility that many patients with a CSF WBC count of >20 cells/μl and a nonreactive CSF-VDRL test result would spontaneously clear their CNS infection without NS treatment. Thus, detecting *T. pallidum* DNA in CSF establishes CNS invasion but not necessarily the degree of CNS involvement.

Many studies evaluated the performance of the FTA-ABS test for the diagnosis of NS, with NPVs ranging from 58.3% to 100% when using comparators having serological evidence of syphilis without NS (16). However, only two of these studies exclusively used HIV-infected patients (31, 32), and only one study exclusively included patients with early syphilis. In our study, the CSF-FTA-ABS test achieved 100% sensitivity for the diagnoses of con-

TABLE 5 Performance characteristics of PCR, FTA-ABS, TPPA, and INNO-LIA assays for the diagnosis of symptomatic and asymptomatic neurosyphilis in HIV-infected patients with early syphilis[a]

Assay	Clinical status[b]	No. of patients	No. of NS cases[c]	Performance characteristics (%)				Likelihood ratios	
				Sensitivity	Specificity	PPV	NPV	Positive	Negative
PCR	S	50	18	61	81	65	79	3.26	0.48
	A	58	6	50	58	12	91	1.18	0.87
FTA-ABS	S	49	18	100	13	40	100	1.15	0
	A	51	5	100	11	11	100	1.12	0
TPPA[d]	S	49	18	72	52	46	76	1.49	0.54
	A	51	4	50	47	7	92	0.94	1.07
INNO-LIA[d]	S	39	15	100	13	42	100	1.14	0
	A	44	5	100	13	13	100	1.15	0

[a] FTA-ABS, fluorescent treponemal antibody absorption; TPPA, *Treponema pallidum* particle agglutination.
[b] S, presence of neurological symptoms; A, absence of neurological symptoms.
[c] NS, neurosyphilis.
[d] Patients with indeterminate results were excluded (TPPA, $n = 3$; INNO-LIA, $n = 10$).

firmed and presumptive NS, but the specificity and positive predictive value (PPV) were low (12% and 25%, respectively). This might be explained by the fact that minimally reactive (1+) FTA-ABS test results were considered positive. Using a higher fluorescence cutoff would have missed 3 confirmed cases of NS. Also, almost a third of our controls had an equivocal CSF WBC count between 6 and 20/μl, a range in which NS cannot be excluded even in HIV-infected patients. Lastly, CSF contamination with blood can be a source of false-reactive results (33), but as mentioned earlier, there was no significant blood contamination in our CSF samples.

Only one previously published study evaluated the TPPA test using CSF samples for the diagnosis of NS (34). The NS definition in that study differed from the one we used: positive microhemagglutination assay for *T. pallidum* antibodies (MHA-TP) and/or FTA-ABS tests, increased number of CSF mononuclear WBCs of >10/μl, and a reactive CSF-VDRL test result. Out of 152 patients with reactive serological test results for syphilis, 133 (88%) were infected with HIV and 16 (11%) had NS according to the diagnostic criteria, and the latter all had reactive CSF-TPPA test results (100% sensitivity). Data from the HIV-infected patients were not presented separately. Among the 77 patients with active untreated syphilis, the specificity of the CSF-TPPA assay for the diagnosis of NS was 65% (50/77). The lower sensitivity (68%) obtained in our study might be explained by our less stringent definition of NS.

The only published study that performed the INNO-LIA Syphilis assay on CSF samples was done on 26 patients suspected to have neurological complications from late-stage syphilis, and 17 of them had a positive test with CSF samples (35). However, this study was not designed to evaluate the performance characteristics of the test in terms of NS diagnosis. While the INNO-LIA assay offered performance characteristics similar to those of the FTA-ABS assay, it is much simpler to perform and does not require fluorescence equipment and expertise. However, its main drawback is the frequency of indeterminate results, which was 20% in our study.

In this study, we showed that PCR, even with its high analytical sensitivity, and the TPPA assay have limited clinical sensitivity and therefore are of limited value for the diagnosis of NS when applied to CSF samples. This is evidenced by their weakly positive likelihood ratios (36). However, the FTA-ABS and INNO-LIA assays were shown to have better sensitivities and thus NPVs that are potentially clinically useful, as evidenced by their strongly negative likelihood ratios. Since the NPV of a test depends not only on its sensitivity and its specificity but also on the prevalence (pretest probability) of the disease in a given population, restricting the analyses to asymptomatic patients improved the NPV, especially for the FTA-ABS and INNO-LIA assays. This might be useful for clinicians following rigorous guidelines that include indications for LPs in asymptomatic patients. Moreover, since symptomatic syphilitic meningitis is usually accompanied by a higher CSF WBC count, a higher CSF protein concentration, and/or a reactive CSF-VDRL test result, assays with high NPVs are most clinically relevant in the subset of asymptomatic patients. Finally, using a CSF WBC cutoff of >10 cells/μl for the diagnosis of NS did not improve the performances of the tests (data not shown).

Our results are limited by the retrospective study design, a potential referral bias, and an important selection bias since many patients were included in the study based on their blood serum RPR titers only. In other words, we did not find any association between neurosyphilis and high RPR titers, but it may simply reflect the fact that most of those who were referred for CSF examination had high RPR titers. Also, since there is no satisfactory gold standard for the diagnosis of NS, we cannot accurately evaluate the specificities of the assays we tested. Lastly, some sensitivity analyses were limited by their small sample sizes.

Conclusion. To our knowledge, this is the most extensive study yet to focus exclusively on the diagnosis of NS in HIV-infected patients with early syphilis. Despite the aforementioned biases, our results suggest that we should not base our decision to perform an LP in HIV-infected patients with early syphilis solely on blood serum RPR titer. However, a CD4 cell count of <500 cells/μl, detectable HIV-1 viremia, headache, and visual symptoms seem more useful for identifying patients who need an LP. Also, we showed that when applied to CSF, the real-time PCR and TPPA assays have limited utility for the diagnosis of NS in a population of HIV-infected patients with early syphilis. We also demonstrated that especially in asymptomatic patients, the FTA-ABS test and the much simpler INNO-LIA Syphilis test might have clinical utility by offering a high NPV, hence allowing one to rule out NS when CSF samples are nonreactive. Even though new technologies are quickly emerging and offer high potential, NS will likely remain a diagnostic challenge for some time.

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