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SHORT COMMUNICATION

Treponema pallidum Haemagglutination Assay Serum Titres as a Predictor of Cerebrospinal Fluid Abnormalities in Patients with Syphilis

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Neurosyphilis can occur at any stage of syphilis infection, even in patients who have received timely and proper treatment (1, 2). The choice of which syphilitic patients should undergo examination of the cerebrospinal fluid (CSF) to diagnose neurosyphilis is difficult. The use of selective lumbar puncture criteria, based on serum titres of non-treponemal tests, have been considered (3–8). We hypothesized that *Treponema pallidum* haemagglutination assay (TPHA) serum titres may also be useful in evaluating the probability of neurosyphilis. In this study we explored the ability of the TPHA to predict basic CSF abnormalities consistent with neurosyphilis (9–12).

MATERIALS AND METHODS

A total of 151 HIV-non-infected patients with serological evidence of syphilis, who had undergone lumbar puncture in the Urals Institute of Dermatovenereology and Immunopathology in Yekaterinburg, Russia, from May 2006 through December 2012, were reviewed.

The patients were part of a consecutive, prospectively collected cohort, and were referred from outpatient clinics for sexually transmitted diseases, neurology units of in-patient hospitals and in-patient psychiatric hospitals. The patients had neurological manifestations consistent with neurosyphilis, late-stage syphilis or a reactive non-treponemal test more than 2 years after treatment for non-neurological syphilis, combined with a low probability of re-infection.

The extensive list of laboratory tests, database maintenance and its use for analysis were approved by the local ethics committee. Written informed consent was provided by all patients. Lumbar puncture was performed in all patients for the first time. Follow-up cases were not included in the study.

Both CSF and blood samples were collected within the same day. CSF samples contaminated with blood were excluded from the study. CSF abnormalities considered consistent with neurosyphilis were: a reactive CSF Venereal Disease Research Laboratory (VDRL) test; CSF white blood cell (WBC) count $> 5/\mu l$; CSF-TPHA titre $\geq 1:640$; TPHA index > 70; intrathecally produced *T. pallidum* antibody (ITpA) index > 3; minimally detectable level of Reiber intrathecal fraction of any immunoglobulin class; and a reactive CSF-FTA-Abs test. Serum TPHA titres were compared across groups with normal and abnormal CSF parameters, and each parameter was tested separately.

A 2-sample Kolmogorov-Smirnov test procedure was used to assess the significance of differences in distributions and to establish a cut-off point. A χ^2 test was used to compare proportions, and Mann-Whitney *U* test to compare continuous and categorical variables. Logistic regression was used to calculate univariate and adjusted odds ratios. All analyses were performed using MedCalc for Windows, version 12.2 (MedCalc Software, Belgium) and Microsoft Excel, version 11.0 (Excel Software, USA).

RESULTS

A total of 151 patients (88 males, 63 females, age range 17–64 years, mean age 40.4 ± 10.5 years) were included in the study. All patients were reactive in more than 2 serum treponemal tests and 133 had reactive serum non-treponemal tests (median rapid plasma reagin (RPR) titre 1:8; range 1:1–1:520).

Thirty-two patients with untreated syphilis had late-stage disease or disease of unknown duration and 2 had secondary syphilis. Five patients were seropositive for syphilis, but were unable to provide information about their previous treatment and were regarded as having late syphilis. A total of 112 patients were treated and re-treated for non-neurological syphilis. The patients received parenteral penicillin (long-, intermediate- or short-acting) and ceftriaxone. The information on serological response to previous treatment was available for 37 patients, of whom 3 had a 4-fold rise and 26 did not have a 4-fold drop in the titre over the recommended follow-up period, and 8 had an adequate serological decline, but the titres did not revert to negative. Seventy-five patients did not have documented information about evolution of non-treponemal test titres; 10 of them showed high titres ($\geq 1:32$), 36 had titres in the range 1:4–1:16, 14 had titres $\leq 1:2$, and 15 were non-reactive. The median (25, 75 percentile) time between initial treatment for syphilis and lumbar puncture was 5 (2.5, 8.7) years.

Eighty-five patients had some clinical symptoms or signs: vision loss ($n=20$), hearing loss ($n=8$), headache ($n=29$), cerebrovascular accident ($n=7$), mental disorders ($n=37$), cranial nerve disorders ($n=55$), motor function disorders ($n=30$), seizures ($n=1$), sensory disorders ($n=5$), coordination disorders ($n=12$) and gait abnormalities ($n=5$). Neurosyphilis was diagnosed based on reactive CSF-VDRL and/or CSF pleocytosis plus reactive CSF-TPHA/CSF-FTA-Abs tests in 40 patients, elevated CSF-TPHA titre/indices and intrathecal synthesis in 4 non-treated patients, and elevated CSF-TPHA titre/indices and intrathecal synthesis plus clinical manifestation and/or high serum non-treponemal titre in 6 treated patients.

The distribution of serum TPHA titers differed significantly between patients with normal and abnormal values for all of the studied CSF parameters ($p<0.0001$) except ITpA index ($p=0.282$). The Kolmogorov-Smirnov test revealed that the best cut-off point was the titer of 1:20480.

CSF abnormalities were more severe and more frequently found in the patients with a serum TPHA titer

≥1:20,480 (Table I). Of the 102 patients with a serum TPHA titer <1:20,480, only 11 (10.7%) met our definition of neurosyphilis, whereas of the 49 patients with a titer ≥1:20,480 39 (80%) patients were diagnosed with neurosyphilis.

After adjusting for serum RPR titre, previous treatment, and duration since treatment, odds ratios for CSF findings did not change significantly (Table SI[1]).

DISCUSSION

Previous studies exploring the usefulness of different serum surrogate markers have shown that patients with neurosyphilis have higher levels of markers compared to syphilis patients without neurosyphilis (3–8, 13, 14).

The present study measured anti-treponemal antibodies and found that a serum TPHA titre ≥1:20,480 was a predictive factor for most CSF abnormalities consistent with neurosyphilis. There was no association between serum TPHA titre and ITpA index. We cannot explain this finding, but it should be taken into consideration when interpreting TPHA results.

De Silva et al. (15) proposed a lower cut-off for serum TPHA titre (1:2,560) when selecting HIV-non-infected late syphilis patients for lumbar puncture. We assume that this was because their study was limited by only positive CSF-TPHA test results (regardless of titre) and that laboratory policy dictated that serum TPHA titrations were not performed beyond a dilution of 1:5,120 in most patients. The authors suggested not performing lumbar puncture in patients with low serum TPHA titres.

Based on our data, we conclude that, although neurosyphilis can be found in patients with any serum TPHA titre, it is significantly more common in those with a titre ≥1:20,480, and such patients are most likely to benefit from CSF examination.

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Table I. *Cerebrospinal fluid (CSF) abnormalities in 151 HIV-non-infected patients with serological evidence of syphilis with serum* Treponema pallidum *haemagglutination assay (TPHA) titres above and below 1:20,480*

	Serum TPHA titre		
CSF abnormalities	<1:20,480 (*n*=102)	≥1:20,480 (*n*=49)	*p*-value
Reactive CSF VDRL, *n* (%)	9 (9)	28 (57)	<0.001[d]
Median, titre (IQR)	1:1 (1:1–1:1)	1:4 (1:2–1:8)	0.0017[e]
CSF WBC count >5/µl, *n* (%)	6 (6)	19 (39)	<0.001[d]
Median (IQR)[a]	7 (7–7)	20 (11–41)	0.008[e]
CSF TPHA titre ≥1:640, *n* (%)	7 (7)	39 (80)	<0.001[d]
Median (IQR)[a]	1:640 (1:640–1:640)	1:2560 (1:1280–1:10,240)	<0.001[e]
TPHA index >70, *n* (%)	8 (8)	39 (80)	<0.001[d]
Median (IQR)[a]	159 (116–193)	689 (329–1351)	<0.001[e]
Intrathecal Ig synthesis, *n* (%)	17 (17)	39 (80)	<0.001[d]
Median, mg/l (IQR)[b]	14.9 (2.2–24.5)	65.1 (24.4–174.4)	<0.001[e]
Reactive CSF FTA-abs, *n* (%)	31 (30)	43 (88)	<0.001[d]
2+, *n*[c]	22	5	
3+, *n*[c]	5	6	<0.001[d]
4+, *n*[c]	4	32	

[a]Calculated only among cases with abnormal CSF values. [b]Total (IgG+IgM+IgA) minimal intrathecally synthesized fraction [13]. [c]Intensity of fluorescence of the treponemes (number of cases). [d]χ^2 test. [e]Mann-Whitney *U* test. IQR: interquartile range.

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