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Elevation of Cerebrospinal Fluid Light and Heavy Neurofilament Levels in Symptomatic Neurosyphilis

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Background: Although clinical manifestations of symptomatic and asymptomatic neurosyphilis are different, few laboratory tests could reflect the difference.

Methods: A total of 92 non-HIV-infected patients with syphilis were enrolled in this study, including 23 with symptomatic neurosyphilis, 51 with asymptomatic neurosyphilis, and 18 with latent syphilis, which were excluded neurosyphilis because they were found to have no symptom and normal cerebrospinal fluid (CSF) tests and served as the control group. The concentrations of neurofilament light subunit (NF-L) and phosphorylated neurofilament heavy subunit (pNF-H) in the CSF were measured and compared among these groups, as well as before and after treatment in the symptomatic and asymptomatic groups.

Results: The median concentrations of NF-L in the symptomatic neurosyphilis, asymptomatic neurosyphilis, and control groups were 5806, 218, and 266 pg/mL, respectively ($P < 0.001$), and the median concentrations of pNF-H were 986, 43, and 49 pg/mL, respectively ($P < 0.001$). A subgroup of 15 symptomatic neurosyphilis and 10 asymptomatic neurosyphilis patients were followed up and underwent CSF examination 6 months after the antineurosyphilis treatment. The median concentration of NF-L in the symptomatic neurosyphilis group decreased from baseline 6420 to 2914 pg/mL after the treatment ($P = 0.03$), and the median concentration of pNF-H in the symptomatic neurosyphilis group decreased from baseline 1399 to 246 pg/mL after the treatment ($P = 0.03$).

Conclusions: Neurofilament light subunit and pNF-H were significantly elevated in the symptomatic neurosyphilis patients, not in asymptomatic neurosyphilis, which was an implication of the different pathogenesis in neurosyphilis.

Over the past decades, the incidence of syphilis has increased from 12.80 per 100,000 individuals in 2006 to 31.97 per

100,000 individuals in 2016 in China.¹ Neurosyphilis is a chronic infectious disease caused by the invasion of *Treponema pallidum* to the central nervous system (CNS). It has been estimated that 4% to 10% of untreated syphilis eventually progresses to neurosyphilis, and the incidence of neurosyphilis is higher in syphilis coinfecting with HIV.²

The nerve filaments, found only in neurons, are sensitive markers of axonal disruption. Nerve microfilaments are released to the cerebrospinal fluid (CSF) in response to injury to myelinated axons.³ Nerve microfilaments are also significantly elevated in several neurodegenerative disorders, including amyotrophic lateral sclerosis, multiple sclerosis, vascular dementia, and Alzheimer disease, which can serve as a candidate biomarker for neurological symptom evaluation and early diagnosis.⁴ The subunits of neurofilaments, such as neurofilament light subunit (NF-L) and phosphorylated neurofilament heavy subunit (pNF-H), are widely used to measure the degree of neuronal damage.^{5,6} There are various clinical manifestations of neurosyphilis. A significant number of neurosyphilis cases present with symptoms of cognitive impairment. In this study, we measured CSF NF-L and pNF-H concentrations in symptomatic and asymptomatic neurosyphilis patients and observed a change in CSF NF-L and pNF-H levels before and after antineurosyphilis treatment in some of these patients.

SUBJECTS AND METHODS

Study Subjects

A total of 92 non-HIV-infected syphilis patients were enrolled in Beijing Ditan Hospital (Beijing, China) from September 2008 to April 2017. Each study participant underwent a standardized clinical assessment of symptoms and signs, blood testing, and lumbar puncture, in which blood and CSF samples were kept at -80°C for detection of NF-L and pNF-H. According to Centers for Disease Control and Prevention guidelines, 23 patients were diagnosed with symptomatic neurosyphilis, who were defined as having CSF abnormalities (reactive CSF venereal diseases research laboratory [VDRL] or CSF white blood cells [WBCs] $>5/\mu\text{L}$) with neurological symptoms, including headache, vision loss, hearing loss, personality changes, impaired memory and judgment, gait incoordination, occasionally seizures, and so on. Participants of the asymptomatic syphilis and control group were enrolled from serofast patients without any symptoms who underwent lumbar puncture, as in our previous study.⁷ Fifty-one patients were diagnosed with asymptomatic neurosyphilis through CSF examination, who were defined as having a reactive CSF VDRL or CSF WBCs $>5/\mu\text{L}$ and absence of neurological symptom. There were 18 latent syphilis patients without any symptom and with normal CSF test results (negative CSF VDRL and CSF WBCs $<5/\mu\text{L}$, normal CSF protein level), who were considered as controls. Patients with HIV-positive or other infection-originated CSF pleocytosis were excluded as well. For patients diagnosed with neurosyphilis, intravenous aqueous penicillin (24 million units per day) divided into 6 doses was used for 14 consecutive days, after which,

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intramuscular injection of benzathine penicillin (2.4 million units per week) was performed for 3 consecutive weeks. Lumbar puncture was repeated in some symptomatic and asymptomatic neurosyphilis patients at 6 months after treatment of neurosyphilis.

The study protocol was reviewed and approved by the ethics committee of Beijing Ditan Hospital, and written informed consent forms were signed by all participants.

Laboratory Examinations

The rapid plasma reagin test was performed according to the routine standard methods as described previously,⁸ and VDRL test was undertaken using the VDRL Antigen Kit (Becton, Dickinson and Company, Franklin Lakes, NJ). The concentrations of NF-L and pNF-H in the CSF were measured by an enzyme-linked immunosorbent assay kit (IBL International [Hamburg, Germany] and BioLegend GmbH [Koblenz, Germany]) according to the manufacturers' instructions. The lower limits of detection were 33 pg/mL (NF-L) and 23.5 pg/mL (pNF-H).

Statistical Analysis

Data were statistically analyzed by SPSS 19.0 software (IBM, Armonk, NY). Normally distributed data were expressed by mean ± SD, and nonnormally distributed data were presented by the median and interquartile range (IQR). The Student *t* test was used to compare the normally distributed data between the 2 groups. The 1-way analysis of variance was used for comparing differences among multiple groups, and the Mann-Whitney *U* test was used for comparing the nonnormally distributed data between the 2 groups. The Pearson correlation analysis and the Spearman rank correlation analysis were used for analysis of correlations between the 2 groups. *P* values ≤0.05 were considered statistically significant. A linear regression (analysis of covariance) was used to model CSF NF-L and pNF-H concentrations in the groups of symptomatic neurosyphilis, asymptomatic neurosyphilis, and controls, adjusted for patient age.

RESULTS

Clinical characteristics of the 92 patients are presented in Table 1. The participants were grouped into symptomatic neurosyphilis

TABLE 1. Demographic Characteristics of the Study Participants

	(n, %) or Median (Interquartile Range)		
	Symptomatic NS (n = 23)	Asymptomatic NS (n = 51)	Uncomplicated Syphilis (n = 18)
Age, y	54 (51–60)	33 (28–46)	32 (27.5–43.3)
Sex			
Male	17 (73.9)	22 (43.1)	8 (44.4)
Female	6 (26.1)	29 (56.9)	10 (55.6)
NS stages			
Meningovascular	6 (26.08)	—	—
General paresis	15 (65.22)	—	—
Tabes dorsalis	1 (4.35)	—	—
Ocular syphilis	1 (4.35)	—	—
1/Serum RPR titer	32 (8–128)	8 (4–16)	4 (1–16)
Reactive CSF VDRL tests	22/23 (95.7)	21/51 (41.2)	0/18 (0)
CSF protein, mg/dL	62 (45.2–88.1)	29.6 (22–38.2)	22.8 (18.78–30.15)
CSF WBC, ×10 ⁶ cells/L	17 (7–50)	10 (6–16)	3 (1.75–4)

RPR indicates rapid plasma reagin.

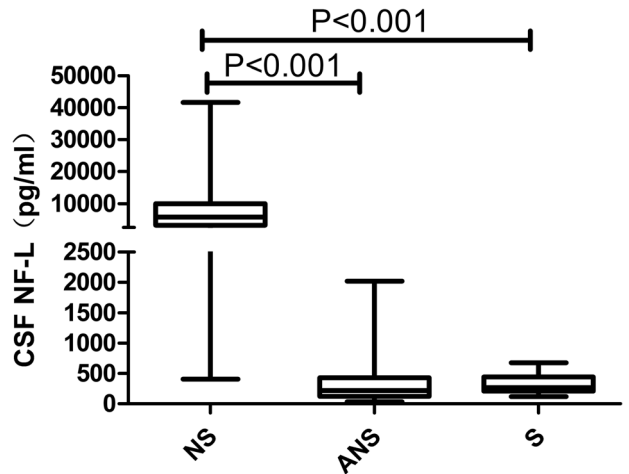


Figure 1. Levels of NF-L in the CSF in the NS, ANS, and nonneurosyphilis control groups. The box plots include median (horizontal line) values from the 25th to the 75th percentiles (boxes). ANS, asymptomatic neurosyphilis; NS, symptomatic neurosyphilis; S, uncomplicated latent syphilis patients.

(n = 23), asymptomatic neurosyphilis (n = 51), and control (n = 18) groups.

Concentrations of NF-L and pNF-H in the CSF

The concentrations of NF-L and pNF-H in the symptomatic neurosyphilis group were significantly higher than those of the asymptomatic neurosyphilis and control groups (*P* < 0.001). The median concentrations of NF-L in the symptomatic neurosyphilis, asymptomatic neurosyphilis, and control groups were 5806 (IQR, 3242–9987), 218 (IQR, 125–430), and 266 (IQR, 209–443) pg/mL, respectively. There was no significant difference in NF-L concentration between the asymptomatic neurosyphilis and control groups (*P* = 0.915; Fig. 1). The median concentrations of pNF-H in the symptomatic neurosyphilis, asymptomatic neurosyphilis, and control groups were 986 (IQR, 342–1799), 43 (IQR, 24–69), and 49 (IQR, 31–67) pg/mL, respectively. There was no significant difference in pNF-H concentration between the asymptomatic neurosyphilis and control groups (*P* = 1.584; Fig. 2).

Among the asymptomatic neurosyphilis patients, according to the diagnostic criteria, there were 21 patients whose CSF VDRL results were positive and 30 patients whose CSF VDRL results were negative. We have compared the concentrations of CSF NF-L and pNF-H in these 2 subgroups; the concentration of NF-L in the CSF VDRL(+) asymptomatic syphilis group was higher than that in the CSF VDRL(–) group, with statistically significance difference (*P* = 0.04), but did not show significant difference as compared with the control group (*P* = 0.811; Fig. 3). There was no significant difference in the CSF pNF-H concentrations among the CSF VDRL(+), CSF VDRL(–) asymptomatic syphilis, and control groups (*P* = 0.394; Fig. 4).

The concentration of NF-L was positively correlated with pNF-H concentration (*r* = 0.764, *P* < 0.001), CSF protein concentration (*r* = 0.635, *P* < 0.001), age (*r* = 0.625, *P* < 0.001), type of neurosyphilis (*r* = 0.564, *P* < 0.001), and CSF VDRL titer (*r* = 0.540, *P* < 0.001). The concentration of pNF-H was positively correlated with type of neurosyphilis (*r* = 0.593, *P* < 0.001), age (*r* = 0.521, *P* < 0.001), CSF VDRL titer (*r* = 0.514, *P* < 0.001), and CSF protein (*r* = 0.541, *P* < 0.001).

Because of unmatched age between the symptomatic neurosyphilis group and the other 2 groups, the analysis of

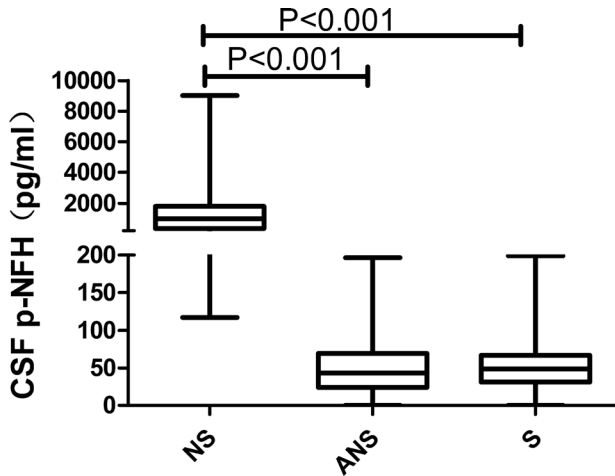


Figure 2. Levels of pNF-H in the CSF in the NS, ANS, and nonneurosyphilis control groups. The box plots include median (horizontal line) values from the 25th to the 75th percentiles (boxes). ANS, asymptomatic neurosyphilis; NS, symptomatic neurosyphilis; S, uncomplicated latent syphilis control.

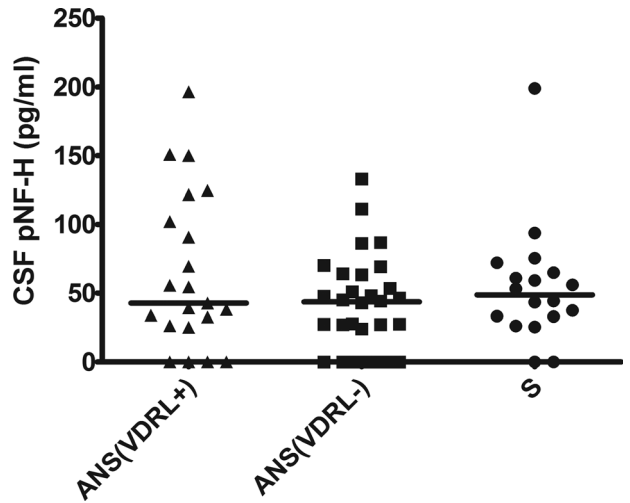


Figure 4. Cerebrospinal fluid pNF-H in VDRL(+)/(–) ANS. Comparison of CSF pNF-H concentration in ANS with a positive or negative CSF VDRL result. ANS, asymptomatic neurosyphilis; S, uncomplicated latent syphilis control.

covariance with NF-L or pNF-H as a dependent variable, disease group as a fixed factor, and age as a covariate was performed. After correcting for age, the difference in CSF NF-L and pNF-H concentrations in the symptomatic neurosyphilis patients remained statistically significant ($F = 14.168$ [$P < 0.01$] and $F = 11.260$ [$P < 0.01$], respectively). The concentrations of CSF NF-L in each group were plotted against age, and CSF NF-L level correlated with age in all 3 groups. When CSF pNF-H concentrations in each group were plotted against age, no linear relationship was shown between CSF pNF-H concentration and age in each group.

Decrease in NF-L and pNF-H Concentrations After Treatment of Neurosyphilis

A subgroup of 15 symptomatic neurosyphilis and 10 asymptomatic neurosyphilis patients were followed up and underwent

CSF examination 6 months after treatment of neurosyphilis (24 million unites of intravenous aqueous penicillin G daily for 14 days). The number of CSF WBCs returned to the normal level, and CSF VDRL became nonreactive in all participants. Some symptoms of symptomatic neurosyphilis patients have resolved after treatment.

The median concentration of NF-L in the symptomatic neurosyphilis group was 6420 (IQR, 3242–17422) pg/mL before treatment, which reduced to 2914 (IQR, 1305–8006) pg/mL after treatment, which was significantly lower than the baseline ($P = 0.03$; Fig. 5). The median concentration of pNF-H in the symptomatic neurosyphilis group was 1399 (IQR, 369–2373) pg/mL before treatment, which decreased to 246 (IQR, 109–472)pg/mL after treatment, which was significantly lower than the baseline ($P = 0.03$;

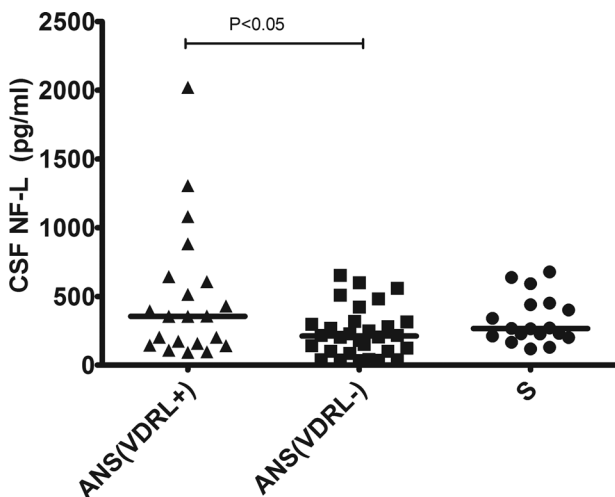


Figure 3. Cerebrospinal fluid NF-L in VDRL(+)/(–) ANS. Comparison of CSF NF-L concentrations in the ANS with a positive or negative CSF VDRL result. ANS, asymptomatic neurosyphilis; S, uncomplicated latent syphilis control.

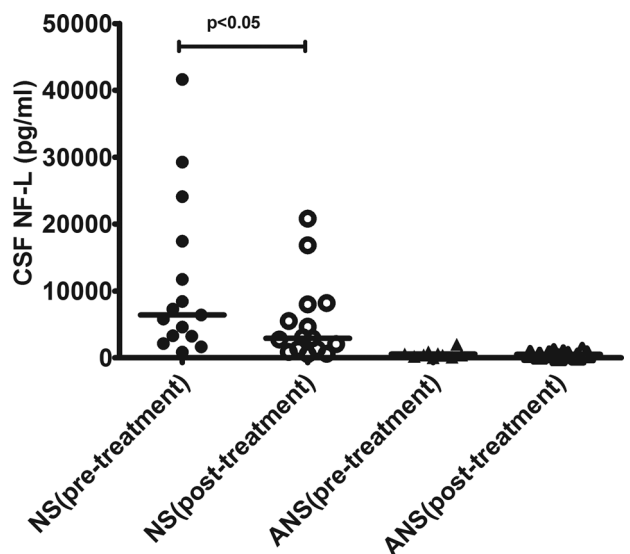


Figure 5. Changes in CSF NF-L levels before and after treatment. Changes in NF-L levels in the CSF in the NS and ANS groups before and 6 months after antineurosyphilis treatment. ANS, asymptomatic neurosyphilis; NS, symptomatic neurosyphilis.

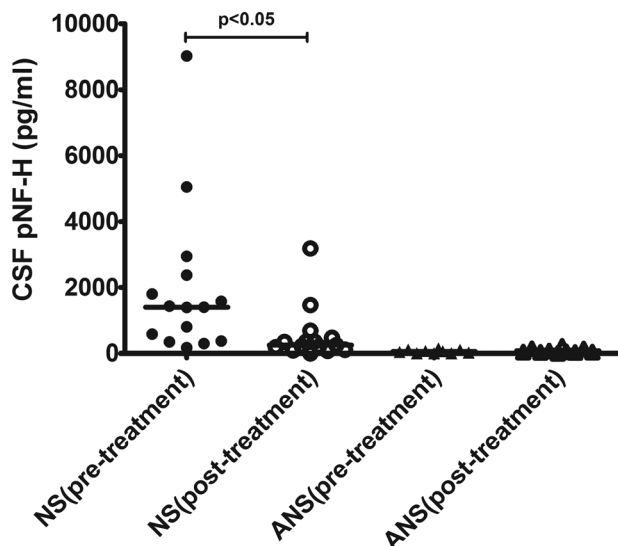


Figure 6. Changes in CSF pNF-H levels before and after treatment. Changes in pNF-H levels in the CSF in the NS and ANS groups before and 6 months after antineurosyphilis treatment. ANS, asymptomatic neurosyphilis; NS, symptomatic neurosyphilis.

Fig. 6). Decreases in NF-L and pNF-H concentrations after treatment of neurosyphilis were only observed in symptomatic neurosyphilis.

DISCUSSION

In the early stage of syphilis infection, *T. pallidum* can invade the CNS in at least 30% of syphilis, whereas only 4% to 10% of the untreated were eventually progressed into symptomatic neurosyphilis.² The pathogenesis of asymptomatic and symptomatic neurosyphilis is still unclear. Diagnosis of neurosyphilis depends on the combination of CSF analysis results and reactive serologic test.⁹ In HIV-uninfected individuals with syphilis, neurological symptoms were independent from the results of CSF VDRL test; neurological symptoms are not associated with a reactive CSF VDRL, nor are these neurological symptoms common in the individuals with a reactive CSF VDRL.¹⁰ In our previous study, we found that there was a correlation between the abnormality of CSF protein levels and the development of symptomatic neurosyphilis, in which the increase in concentration of CSF protein in symptomatic neurosyphilis compared with that of asymptomatic neurosyphilis was statistically significant.⁸ In this study, the concentrations of both NFL and pNF-H were substantially increased in the CSF in symptomatic neurosyphilis rather than in asymptomatic neurosyphilis. Our results implicated the difference in neuronal damage between symptomatic and asymptomatic neurosyphilis.

Both NF-L and pNF-H are subunits of neurofilament protein, which is only found in the cytoplasm of neurons, and they are highly specific biomarkers for myelinated axons. In case of damage to neurons, filament protein may be released into the CSF.³ In addition, NF-L and pNF-H are widely used as indicators to measure the extent of damage to CNS in other neurodegeneration diseases.^{5,6} A significant number of patients with neurosyphilis present with dementia with a progressive course and psychiatric symptoms such as depression, mania, and psychosis.¹¹ In this study, we have demonstrated that there were significant differences in the concentration of CSF NF-L and pNF-H, which indicate different pathogenesis between symptomatic neurosyphilis and asymptomatic neurosyphilis.

Ho et al.¹² found no correlation in CSF NF-L concentration between HIV-infected patients with neurosyphilis and uncomplicated syphilis. In their study, symptomatic neurosyphilis and asymptomatic neurosyphilis were not distinguished according to the including criteria of the neurosyphilis groups, which could underestimate the significance of CSF NF-L elevation in symptomatic neurosyphilis. The diagnosis of asymptomatic neurosyphilis is solely based on CSF test, including reactive CSF VDRL or CSF pleocytosis. We have compared the concentrations of NF-L and pNF-H of CSF VDRL(+) and VDRL(-) subgroups; they were not significantly different as compared with the control group. Interestingly, the minimum concentration of NF-L was 409 pg/mL in symptomatic neurosyphilis; the majority (21/23; 91%) had high NF-L concentrations of >1000 pg/mL. In contrast, none had high NF-L concentrations in the control group. However, 6% (3/51) of asymptomatic neurosyphilis patients had NF-L concentrations greater than 1000 pg/mL, and all these 3 patients had positive a CSF VDRL result. The aforementioned results have an important implication of brain damage before the symptoms appeared in some of the CSF VDRL-positive asymptomatic neurosyphilis patients.

Our finding of a positive association between CSF NF-L and age is in agreement with a previously reported study.¹³ In our study, the participants of the asymptomatic neurosyphilis and control groups were enrolled from serofast patients who needed to have a CSF test. The average age of these 2 groups was lower than that of the symptomatic neurosyphilis group. After correcting for age, the difference in CSF NF-L and pNF-H concentration remained statistically significant.

After neurosyphilis treatment, concentrations of NF-L and pNF-H were markedly decreased in symptomatic neurosyphilis, suggesting that levels of NF-L and pNF-H could be changed in responses to the treatment. Although CSF NFL and pNF-H concentrations did not return to control concentrations after 6 months of treatment, it is possible that these concentrations will decrease over time, and these patients are still in further follow-up. There are 2 possible outcomes, one of which is the return of CSF concentration to the control group level and the other is when CSF concentration is still higher than the control group level for a considerable period of time, reflecting different pathogenic mechanisms and clinical outcomes, and these patients are still following up. This finding indicated that neuron damage caused by syphilis could recover after treatment. The clinical significance of NF-L and pNF-H concentration decline requires further study in the future.

Limitation of this study is having relatively small, age-unmatched groups. Such discrepancy between age of the symptomatic neurosyphilis group and other 2 groups simply reflects the reality that symptomatic neurosyphilis and asymptomatic neurosyphilis were diagnosed in different stages of infection. Most symptomatic neurosyphilis cases were not found until many years after infection. We tried to counterbalance by adjusting for age in statistical analysis. Age factors did not have a significant impact on the results in this study. However, further investigation is needed to evaluate the roles of CSF NF-L and pNF-H in the pathogenesis of neurosyphilis by a longitudinal cohort study.

In conclusion, we confirmed that the concentration of CSF NF-L and pNF-H in symptomatic neurosyphilis increased, which was an important implication of different pathogenesis in neurosyphilis.

REFERENCES

1. Yang S, Wu J, Ding C, et al. Epidemiological features of and changes in incidence of infectious diseases in China in the first decade after the

- SARS outbreak: An observational trend study. *Lancet Infect Dis* 2017; 17:716–725.
2. Ghanem KG. REVIEW: Neurosyphilis: A historical perspective and review. *CNS Neurosci Ther* 2010; 16:e157–e168.
 3. Yuan AD, Rao MV, Veeranna, et al. Neurofilaments and neurofilament proteins in health and disease. *Cold Spring Harb Perspect Biol* 2017; 9:a018309.
 4. Perrot R, Berges R, Bocquet A, et al. Review of the multiple aspects of neurofilament functions, and their possible contribution to neurodegeneration. *Mol Neurobiol* 2008; 38:27–65.
 5. Gresle MM, Butzkueven H, Shaw G. Neurofilament proteins as body fluid biomarkers of neurodegeneration in multiple sclerosis. *Mult Scler Int* 2011; 2011:315406.
 6. Pasol J, Feuer W, Yang C, et al. Phosphorylated neurofilament heavy chain correlations to visual function, optical coherence tomography, and treatment. *Mult Scler Int* 2010; 2010:542691.
 7. Cai SN, Long J, Chen C, et al. Incidence of asymptomatic neurosyphilis in serofast Chinese syphilis patients. *Sci Rep* 2017; 7:15456.
 8. Wei CB, Lun WH, Wan G, et al. Comparison of the symptomatic and asymptomatic neurosyphilis patients for clinical and laboratory features. *Chin J Exp Clin Infect Dis* 2013; 28:59–62.
 9. Workowski KA, Bolan GA, Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recomm Rep* 2015; 64:1–137.
 10. Davis AP, Stern J, Tantalò L, et al. How well do neurologic symptoms identify individuals with neurosyphilis. *Clin Infect Dis* 2018; 66:363–367.
 11. Keskin G, Sunter G, Midi I, et al. Neurosyphilis as a cause of cognitive decline and psychiatric symptoms at younger age. *J Neuropsychiatry Clin Neurosci* 2011; 23:E41–E42.
 12. Ho EL, Maxwell CL, Dunaway SB, et al. Neurosyphilis increases human immunodeficiency virus (HIV)-associated central nervous system inflammation but does not explain cognitive impairment in HIV-infected individuals with syphilis. *Clin Infect Dis* 2017; 65:943–948.
 13. Vagberg M, Norgren N, Dring A, et al. Levels and age dependency of neurofilament light and glial fibrillary acidic protein in healthy individuals and their relation to the brain parenchymal fraction. *PLoS One* 2015; 10:e1035886.