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ORIGINAL RESEARCH

# Analysis of *Treponema pallidum* DNA and CXCL13 in Cerebrospinal Fluid in HIV-Negative Syphilis Patients

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**Purpose:** Neurosyphilis (NS) is a chronic infectious disease associated with *Treponema pallidum* subsp. *pallidum* (TP) infection of the central nervous system. The purpose of this study was to offer evidence for the diagnosis and treatment of NS by revealing the detection of TP DNA and CXCL13 concentration in the cerebrospinal fluid (CSF) of HIV-negative syphilis patients.

**Patients and Methods:** This study included 75 syphilis patients. The frequency of TP invasion into the CSF was detected by nested PCR. ELISA was performed to detect CSF CXCL13 concentrations, and ROC analysis was performed to assess diagnostic accuracy. Sociodemographic data, clinical symptoms, and laboratory indices of patients were collected. CSF CXCL13 levels and clinical characteristics of syphilis patients were investigated retrospectively.

**Results:** The detection rate of CSF DNA of TP by nested PCR was 5.3% and 16.7% in HIV-negative syphilis patients and NS patients, respectively. There was a significant difference between the NS and non-NS groups in terms of neurological symptoms, CSF TPPA, CSF TRUST, CSF nucleated cells, CSF protein, and CSF CXCL13 levels ($P<0.05$). ROC curve analysis showed that the AUC for CSF CXCL13 levels was 0.906 (95% CI 0.832–0.981, $P<0.0001$), with an optimal critical value of 57.85 pg/mL and sensitivity and specificity of 88.89% and 78.95%, respectively.

**Conclusion:** Nested PCR can be used as an auxiliary diagnosis of NS, and CSF CXCL13 >60 pg/mL has high sensitivity and specificity for NS patients and non-NS patients. CXCL13 may be a useful marker to distinguish NS from non-NS syphilis in HIV-negative patients.

**Keywords:** neurosyphilis, *Treponema pallidum*, cerebrospinal fluid, CXCL13

## Introduction

Syphilis is a chronic infectious disease caused by infection with *Treponema pallidum* subsp. *pallidum* (TP), of which humans are the only known hosts. Neurosyphilis (NS) is a series of syndromes caused by the invasion of TP into the brain parenchyma or meninges, which are mainly present as neurological abnormalities. The clinical manifestations of NS are varied. Previously, NS was considered to be a lesion of late syphilis, but recent studies have found that NS can occur at any stage of the disease course in syphilis patients.[1] Diagnosing NS in clinical work remains a complex issue, and the currently accepted diagnostic criteria are based on a combination of clinical manifestations and laboratory findings. There is no gold standard for the diagnosis of NS, and timely diagnosis and treatment of these patients by clinicians remains a great challenge. Therefore, it is important to explore additional cerebrospinal fluid (CSF) tests and biomarkers to help diagnose NS. The completion of the whole genome sequencing of the TP Nichols strain laid the foundation for the molecular study of syphilis spirochetes.[2] Detection of TP DNA in the CSF of syphilis patients by nested polymerase chain reaction (nPCR) gives an idea of the frequency of TP invasion into the CSF. The production of cytokines and chemokines may help in the diagnosis of NS and in tracking the progression of the disease. CXCL13 is

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a member of the CXC chemokine family. It is mainly produced by follicular dendritic cells and is involved in central nervous system injury via chemotactic B cells.[3] CXCL13 does not enter the CSF with the blood circulation, even when the blood-brain barrier is severely dysfunctional, so the cause of elevated CSF CXCL13 concentrations may originate directly in the central nervous system.[4] By identifying TP DNA and CXCL13 concentrations in the CSF of syphilis patients who tested negative for the human immunodeficiency virus (HIV), we were able to study their clinical characteristics. CXCL13 levels were then utilized to assess the possibility of NS in syphilis patients, serving as a foundation for NS prediction and efficacy evaluation.

# Methods

## Diagnostic Criteria

The diagnosis of NS is based on the guidelines of the US Centers for Disease Control and Prevention, the recommendations of Marra, and the latest surveillance cases.[5–9] The diagnostic criteria for high suspicion of NS developed in this study included a diagnosis of syphilis confirmed by history, clinical examination, and laboratory tests, and one of the following three conditions: (1) presence of neurological symptoms or signs and positive CSF TPPA; (2) presence of neurological symptoms or signs and CSF protein >450 mg/L and CSF nucleated cell count >5/uL without other known causes; (3) absence of neurological symptoms or signs and a positive CSF TPPA and CSF protein >450 mg/L and CSF nucleated cell count >5/uL without other known causes. Early symptomatic NS common neurological symptoms include headache, memory decline, neck stiffness, nausea, vomiting, seizures, hemiparesis, and optic or auditory neuropathy. Common symptoms in the late NS include progressive dementia, psychotic symptoms, personality change, manic delusions, and gait abnormalities.[1,10] Among them, we classified those meeting the conditions of (1) and (2) as symptomatic NS and those meeting the conditions of (3) as asymptomatic NS. The syphilis stages were classified as early (primary, secondary, and early latent) or late (tertiary and late latent) according to the clinical examination, history, and infection time.[5,11]

## Study Participants

This study was conducted in the Department of Dermatology and Venereal Disease of the First Affiliated Hospital of Guangxi Medical University in southern China. We included a total of 75 syphilis patients who underwent lumbar punctures from January 2021 to July 2022. During the study period, we excluded patients with HIV infection, intracranial infection, or other intracranial diseases, and we excluded CSF samples from patients with visible blood contamination or duplication. In our study, the subjects were from the following syphilis patients who underwent CSF examination: (1) developed neurological symptoms or signs without other known causes of the clinical abnormalities; (2) had syphilis with a history of more than 2 years and/or no regular treatment.

After patients' informed consent, we collected non-anticoagulated serum samples for the serum syphilis spirochete particle agglutination test (TPPA) and toluidine red unheated serum test (TRUST), performed lumbar puncture, and collected CSF for CSF nucleated cells, CSF protein, CSF TPPA, and CSF TRUST assays. Their clinical data and laboratory information were collected through the hospital's electronic medical record system.

## Laboratory Testing

Serological testing for syphilis using TRUST (Rongsheng, China) and TPPA (Fuji, Japan) was performed on all samples. 2–4 mL of CSF was retained for timely storage at −80°C for TP nPCR detection and the CXCL13 assay. CSF CXCL13 concentrations were measured by enzyme immunoassay using the Human CXCL13 Simple Step Elisa kit (Abcam, UK), with 50 uL CSF added to each kit. All of the above tests were performed according to the manufacturer's instructions. CSF samples were diluted as necessary to meet the analytical range. DNA was extracted from CSF using QIAamp DNA Mini Kits (Qiagen, Germany). nPCR was performed using primers targeting the tpp47 gene (GenBank: QCQ80232.1), which are shown in Table 1.[12] We used a Nanodrop spectrophotometer to detect the concentration of extracted DNA. The internal primers are expected to amplify a 261bp DNA fragment, and nPCR products were analyzed by electrophoresis using a 3% agarose gel. The results were analyzed with a gel imager.

**Table 1** Primer Sequences for nPCR Amplification of TPP47 Gene

| | Primer Name | Primer Sequences |
|---|---|---|
| External primers | 47KDa-1 | 5′- GTTGAGGTATTGGGCGAAAA-3′ |
| | 47KDa-2 | 5′- ATACCCGTTCGCAATCAAAG-3′ |
| Internal primers | 47KDa-3 | 5′- GAAGTTTGTCCCAGTTGCGGTT-3′ |
| | 47KDa-4 | 5′- CAGAGCCATCAGCCCTTTTCA-3′ |

**Note**: Data from Palmer et al.[12]

## Statistical Analysis

SPSS 17.0 (IBM SPSS software, Armonk, NY, USA) was used for analyze the statistical data and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA) was used for plotting. Normally distributed data is described as mean ± standard deviation, and skewed data is described using median and interquartile spacing. The Mann–Whitney *U*-test or ANOVA was used for continuous variables with a skewed distribution, and the $\chi^2$ test or Fisher's exact test was used for categorical variables. P<0.05 indicated statistical significance between two groups of variables. Receiver operating characteristic (ROC) analysis was performed to determine the performance of CSF CXCL13 content, and the optimal cutoff value was determined based on the highest Youden index (sensitivity + specificity - 100%).

## Ethics Approval and Consent to Participate

The study was conducted in accordance with the ethical standards of the Declaration of Helsinki and has been approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, China (ethical approval no. 2022-S005-01). All participates were received and signed informed consent.

# Results

## Patient Characteristics

Of these 75 cases, 35 (46.7%) patients had early syphilis, 30 (40.0%) patients had late syphilis, and 10 (13.3%) patients were clinically undetermined. These 10 unknown cases were due to confusing medical histories or a lack of previous medical records and historical laboratory results to support the diagnosis. Of the 75 patients with syphilis, 37 (49.3%) were male and 38 (50.7%) were female. The prevalence of NS is 24.0% (n=18). Of the 18 patients with NS, 15 (83.3%) had symptomatic NS and 3 (16.7%) had asymptomatic NS (Table 2). Among them, CSF CXCL13 levels were significantly higher in the NS group than in the non-NS group. There were significant differences between NS group and non NS group in neurological symptoms, CSF TPPA, CSF TRUST, CSF nucleated cells and CSF protein (P<0.05).

**Table 2** General Characteristics of 18 NS Patients

| Case | Gender (F/M) | Age (Year) | Neurological Symptoms or Signs | Serum TRUST | CSF TPPA | CSF Protein (mg/L) | CSF Nucleated Cell (Cells/uL) | CSF CXCL13 (pg/mL) |
|---|---|---|---|---|---|---|---|---|
| 1 | M | 49 | – | 1:16 | + | 672.6 | 146 | 57,262.0 |
| 2 | F | 53 | Memory decline | 1:64 | + | 645.7 | 28 | 12,245.21 |
| 3 | M | 27 | Memory decline, seizures | 1:2 | + | 546.4 | 10 | 5445.25 |
| 4 | M | 37 | Memory decline | 1:2 | + | 455.5 | 2 | 112.0 |
| 5 | M | 66 | Hemiparesis, memory decline | 1:16 | + | 1095.7 | 10 | 1540.14 |
| 6 | F | 68 | Hemiparesis | 1:1 | + | 1198.1 | 6 | 2738.86 |
| 7 | M | 34 | Personality change | 1:1 | + | 185.1 | 8 | 1709.24 |
| 8 | M | 37 | Memory decline | 1:16 | + | 505.5 | 10 | 1282.75 |
| 9 | F | 58 | Blurred vision | 1:2 | – | 994.4 | 1080 | 980.02 |
| 10 | F | 34 | Headache | 1:2 | + | 483.6 | 0 | 153.54 |

(*Continued*)

**Table 2** (Continued).

| Case | Gender (F/M) | Age (Year) | Neurological Symptoms or Signs | Serum TRUST | CSF TPPA | CSF Protein (mg/L) | CSF Nucleated Cell (Cells/uL) | CSF CXCL13 (pg/mL) |
|---|---|---|---|---|---|---|---|---|
| 11 | F | 45 | Headache, vomit | 1:4 | + | 248.7 | 0 | 62.05 |
| 12 | M | 48 | Hemiparesis | 1:8 | – | 1193.6 | 15 | 58.22 |
| 13 | M | 58 | Gait abnormalities | 1:128 | + | 834.6 | 56 | 143.55 |
| 14 | M | 17 | Seizures | 1:128 | + | 276.5 | 5 | 40.68 |
| 15 | F | 32 | – | 1:4 | + | 800.6 | 36 | 35.10 |
| 16 | M | 31 | Hemiparesis | 1:16 | + | 470.0 | 32 | 5835.75 |
| 17 | F | 28 | – | 1:64 | + | 458.1 | 140 | 16,557.65 |
| 18 | M | 46 | Headache, memory decline | 1:64 | + | 1285.1 | 180 | 3469.0 |

**Abbreviations**: M, Male; F, Female; –, No neurological symptoms or signs.

In contrast, there were no significant differences in age, gender, serum TRUST titers and CSF nPCR between the two groups ($p>0.05$) (Table 3).

## Low Detection Rate of Treponema Pallidum DNA in CSF

We used nPCR to detect TP DNA in the CSF of 75 HIV-negative syphilis patients, 4 cases were positive (Table 4). Thus, the detection rates of TP DNA in the CSF of HIV negative syphilis patients and NS patients were 5.3% (4/75)

**Table 3** Characteristic of the Study Population

| | Neurosyphilis (n=18) | Non-Neurosyphilis (n=57) | $t/\chi^2/z$ | P value |
|---|---|---|---|---|
| Age (Year) | 41 (17–68) | 34 (12–76) | 1.741 | 0.086 |
| Gender | | | 1.314 | 0.252 |
| Female (%) | 7 (38.9) | 31 (54.4) | | |
| Male (%) | 11 (61.1) | 26 (45.6) | | |
| Neurological symptoms (%) | 15 (83.3) | 8 (14.0) | 30.897 | 0.000 |
| Serum TRUST titer (median) | 1:12 (1:1–1:128) | 1:2 (negative[a]-1:64) | −1.766 | 0.077 |
| CSF nucleated cells ($\times10^6$/L) | 12.5 (0–1080) | 2 (0–30) | −4.545 | 0.000 |
| CSF protein (mg/L) | 596.1 (185.1–1285.1) | 297.1 (152.1–527.0) | −4.627 | 0.000 |
| No. (%) CSF TRUST positive | 11 (61.1) | 0 | 36.084 | 0.000 |
| No. (%) CSF TPPA positive | 16 (88.9) | 9 (15.8) | 32.895 | 0.000 |
| CSF CXCL13 Concentrations (pg/mL) | 1411.4 (35.1–57,262.0) | 34.6 (2.3–4101.7) | −5.173 | 0.000 |
| No.(%) CSF nucleated cells> 5 ($\times10^6$/L) | 14 (77.8) | 13 (22.8) | 17.942 | 0.000 |
| No.(%) CSF protein> 450(mg/L) | 15 (83.3) | 5 (8.8) | 38.891 | 0.000 |
| No. (%) CSF CXCL13> 57.85 (pg/mL) | 16 (88.9) | 12 (21.1) | 24.086 | 0.000 |
| No. (%) CSF nPCR positive | 3 (16.7) | 1 (1.8) | 3.434 | 0.064 |

**Note**: [a]12 patients with negative serum TRUST.

**Table 4** General Characteristics of CSF TP DNA Positive Patients

| Case | Gender (F/M) | Age (Year) | Serum TRUST | CSF TPPA | CSF TRUST | CSF Protein (mg/L) | CSF Nucleated Cells (Cells/uL) | CSF CXCL13 (pg/mL) | Diagnosis |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 49 | 1:16 | + | 1:1 | 672.6 | 146 | 57,262.0 | Late NS |
| 2 | M | 37 | 1:16 | + | 1:2 | 505.5 | 10 | 1282.75 | Early NS |
| 3 | F | 34 | 1:2 | – | – | 152.1 | 2 | 16.02 | Late latent syphilis |
| 4 | F | 58 | 1:32 | – | – | 994.4 | 1080 | 980.02 | Early NS |

**Abbreviations**: M, Male; F, Female; –, Negative; +, Positive.

and 16.7% (3/18), respectively. Among the 4 positive cases, 2 were male, 2 were early NS,1 was late NS and 1 was late latent syphilis.

## CSF CXCL13 Levels Were Significantly Higher in NS Patients Than in Non-NS Patients

To determine CSF CXCL13 levels in syphilis patients, we performed a quantitative ELISA analysis and tested the CSF of 75 syphilis patients. CSF CXCL13 levels in patients with NS were approximately 40.8 times higher than those in non-NS patients, with median CXCL13 levels of 1411.44 pg/mL (35.1 pg/mL–57,262.0 pg/mL) and 34.6 pg/mL (2.3pg/mL–4101.7 pg/mL) in the two groups, respectively. We performed receiver operating characteristic (ROC) analysis and the corresponding AUC values (area under the ROC curve) to assess the sensitivity of CSF CXCL13 concentrations to discriminate between these two groups of syphilis patients (Figure 1). The results showed that the AUC for CSF CXCL13 levels was 0.906 (95% CI 0.832–0.981, P< 0.0001), with an optimal cut-off value of 57.85 pg/mL and sensitivity and specificity of 88.89% and 78.95%, respectively.

## Characteristics of Non-NS Patients with High CSF CXCL13 Concentrations

In our study, 12/57 (21.1%) non-NS patients had CSF CXCL13 concentrations greater than the threshold value, ie, CSF CXCL13 >57.85 pg/mL. Only 1 of these patients had elevated CSF protein, but 6 had elevated cells. The characteristics of these 12 patients are summarized below (Table 5) which included a total of 4 males and 8 females with a mean age of 31.3 years, a mean TRUST serum titer of 1:32, and a median CSF CXCL13 concentration of 138.93 pg/mL (58.51 pg/mL–4101.70 pg/mL). The CSF CXCL13 concentration in the NS group was approximately 10.2 times higher than in this group of patients.

# Discussion

Compared to conventional PCR, nPCR is more specific and sensitive, which can improve the accuracy of amplified products. Our results showed that the detection rate of CSF DNA of TP by nPCR was not high in HIV-negative syphilis patients and NS patients; it was 5.3% and 16.7%, respectively. Our results showed that TP was detected in the CSF of patients in the early and late stages of syphilis, which is consistent with previous studies.[1,6] Interestingly, we found TP DNA in the CSF of a patient with late latent syphilis who had not yet met diagnostic criteria for NS and had a low CSF CXCL13 concentration. However, we were unable to determine whether our patient presented with a potential persistent infection or relapse. A study indicated that the positive rate of TP PCR in the CSF of patients with primary and secondary syphilis was 28.6%.[13] Other relevant reports showed that the sensitivity of nPCR for detecting CSF TP was 42.5% and 27%, and the specificity was 97% and 100%, respectively.[14,15] The lower sensitivity of our results may be due to the

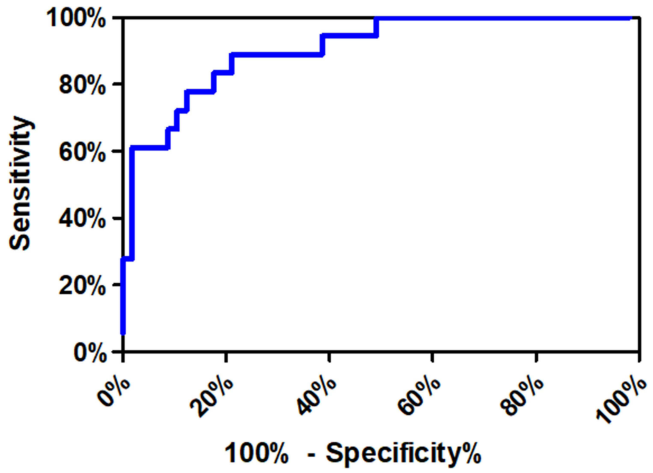


**Figure 1** Subject operating characteristic (ROC) curve analysis of CSF CXCL13.

**Table 5** Characteristics of Non-NS Patients with High CXCL13 Concentrations

| Case | Gender (F/M) | Age (Year) | Serum TRUST | CSF TPPA | CSF Protein (mg/L) | CSF Nucleated Cell (Cells/uL) | CSF CXCL13 (pg/mL) |
|---|---|---|---|---|---|---|---|
| 1 | F | 42 | – | – | 451.3 | 5 | 83.33 |
| 2 | F | 20 | 1:32 | – | 252.7 | 0 | 89.06 |
| 3 | M | 24 | 1:64 | – | 343.1 | 10 | 94.07 |
| 4 | F | 56 | 1:32 | – | 305.7 | 14 | 464.87 |
| 5 | F | 28 | 1:64 | + | 407 | 9 | 797.13 |
| 6 | F | 31 | 1:64 | – | 258.8 | 6 | 150.28 |
| 7 | M | 25 | 1:4 | + | 371.2 | 0 | 58.51 |
| 8 | F | 31 | 1:1 | – | 259.1 | 2 | 58.80 |
| 9 | M | 22 | 1:2 | + | 255.8 | 0 | 160.76 |
| 10 | F | 34 | – | – | 428.0 | 0 | 740.25 |
| 11 | M | 43 | 1:32 | + | 223.2 | 8 | 127.58 |
| 12 | F | 19 | 1:64 | – | 298.6 | 30 | 4101.70 |

**Abbreviations**: M, Male; F, Female; –, Negative; +, Positive.

difference in the study population, whereas Vanhecke's study included HIV-infected as well as severely immunodeficient syphilis patients, which can exacerbate NS infection.[14] The nPCR test may not be the most sensitive method to diagnose NS. It should be noted, however, that CSF TP DNA detection can provide direct evidence of TP invading the nervous system.

The non-treponemal tests of CSF mainly include the venereal disease research laboratory (VDRL) test, the rapid plasma reagin (RPR) test and the TRUST test.[16] A meta-analysis showed that the sensitivity of the CSF-VDRL ranged from 49–87.5% and the specificity ranged from 74–100% for diagnosing NS. CSF RPR is 51–82% sensitive and 82–100% specific for diagnosing NS.[17] A study reported a sensitivity of 76.2% and specificity of 93.1% for CSF TRUST.[7] The CSF-VDRL is currently considered the definitive test for the diagnosis of NS worldwide. However, the test has several limitations, including its moderate sensitivity, lack of commercial availability in resource-limited countries, and cumbersome and time-consuming procedures. Blood contamination of CSF may cause false positives for non-treponemal tests in the CSF of syphilitic patients.

The treponemal tests mainly include the fluorescent treponemal antibody absorption (FTA-ABS) test, the Treponema pallidum haemagglutination assay (TPHA), the TPPA test, and enzyme immunoassay (EIA). Marra has reported that the sensitivity of the CSF FTA-ABS test is 98.3% and the specificity is 100%.[18] A study showed that CSF TPPA test sensitivity is 86.2–100%, and the specificity ranges from 99.6 to 100%.[19] Since the anti-treponemal IgG antibody can cross the blood-brain barrier and enter the CSF, it may cause false positives in treponemal tests. The CSF treponemal test plays an important role in the diagnosis of NS and has high sensitivity but relatively low specificity. The Chinese guidelines take the CSF FTA-ABS test results as a diagnostic indicator of neurosyphilis, and point out that the CSF TPPA test can be used to replace the CSF FTA-ABS test in the absence of these conditions. The positive results need to be evaluated in combination with clinical manifestations, CSF nucleated cells, and proteins to better diagnose NS.

The diagnosis of NS is not difficult when the patient has typical signs and symptoms. In contrast, patients with asymptomatic NS lack neurological symptoms and have only abnormal CSF tests. It is necessary to combine more sensitive indicators for NS diagnosis. Therefore, we evaluated CSF CXCL13 as a biomarker for the diagnosis of NS in HIV-negative NS. A study reported that the CSF CXCL13 concentration is particularly useful in the diagnosis of NS in HIV-infected patients because it is independent of CSF polycythemia vera and HIV markers and decreases after treatment.[20] In our study, we found significantly higher CSF CXCL13 concentrations in NS patients than in non-NS patients ($p<0.05$), which further strengthens the previous findings. The median CSF CXCL13 concentration in NS patients was 1411.44 pg/mL, which was 40.8 times higher than that in non-NS patients. Our results are high in comparison to the previously reported median of 972 pg/mL.[21] It was possible that there were differences in the

disease activity. In addition, those differences in the manufacturers of the reagents used for testing may have contributed to the bias.

ROC analysis showed that CSF CXCL13 levels were very reliable in distinguishing NS from non-NS, with an AUC of 0.906 and a cut-off value of 57.85 pg/mL, with sensitivity and specificity of 88.89% and 78.95%, respectively. Compared with the study of Zeng, our specificity was similar, and the cut-off value and sensitivity were higher.[22] Mothapo suggested using a cut-off of 76.3 pg/mL with a sensitivity and specificity of 50% and 90%, respectively, in differentiating NS from non-NS patients.[23] Marra showed that a threshold value of 250 pg/mL was highly specific for the diagnosis of symptomatic and asymptomatic NS.[20] Our cut-off value is relatively low because the study populations in Mara and Mothapo included syphilis patients infected with HIV. HIV can promote the development of syphilis infection and can lead to CSF nucleocytosis. So, compared to HIV-negative syphilis infection, HIV co-infection with syphilis may encourage greater CSF CXCL13 production, with a correspondingly higher threshold. Moreover, studies showed that the serum TRUST titer, CSF TPPA, CSF TRUST, CSF B cells, CSF nucleated cells, CSF protein, and CSF IgG index showed a significant positive correlation with CXCL13 and a negative correlation with IL4.[24–26] Marra also emphasized that increasing CSF CXCL13 concentrations increased the risk of developing NS significantly.[19] The level of CXCL13 may be a potential assessment of disease activity for NS.

It is important to recognize that our research has some limitations. First off, although we strictly control the diagnostic criteria of NS, we only discussed HIV negative syphilis patients. We were unable to compare the CSF results between the NS group, the general syphilis group, and healthy controls since lumbar puncture was not conducted in healthy individuals. Second, there are inevitable biases in the concentrations measured by CXCL13 kits from different manufacturers or different brands, so a large amount of data is needed to standardize the diagnostic cut-off. Third, the follow-up period for patients with common syphilis with high CSF CXCL13 is insufficient, and whether this population is at high risk of developing NS remains to be determined longitudinally. Due to considerable population mobility, poor patient compliance, and a lack of follow-up data in our institution, we were unable to establish a reliable prediction. To further support our findings in syphilis patients, we require additional research with sizable sample sizes.

## Conclusion

The detection rate of CSF TP by nPCR was 5.3% and 16.7% in HIV-negative syphilis patients and NS patients, respectively. In our study, CSF CXCL13 levels were significantly elevated in patients with NS. CSF CXCL13 had high sensitivity, specificity, and AUC. CXCL13 may be a potential marker for the diagnosis of NS activity. Further follow-up studies are needed to evaluate the predictive value of CXCL13.

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## Disclosure

The authors report no conflicts of interest in this work.

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