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CXCL13 and TH1/Th2 cytokines in the serum and cerebrospinal fluid of neurosyphilis patients

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Abstract

Neurosyphilis is a chronic infectious disease with involvement of central nervous system infection by *Treponema pallidum*. This study was to investigate the contents of B lymphocyte chemokine 1 (BLC-1/chemokine [C-X-C motif] ligand 13), Th1 cytokines (Interleukin [IL]-2, IL-12, and Interferon [IFN]-γ), and Th2 cytokines (IL-6 and IL-10) in serum and cerebrospinal fluid (CSF) of HIV-negative patients with neurosyphilis before and after treatment, aiming to elucidate roles of CXCL13 and Th1/Th2 cytokines in immune response to and pathogenesis of neurosyphilis.

Enzyme-linked immunosorbent assay was employed to detect the contents of CXCL13, IL-2, IL-12, IFN-γ, IL-6, and IL-10 in serum and CSF of 47 HIV-negative patients with neurosyphilis, 36 syphilis patients without neurological involvement and 23 controls (noninfectious intracranial disease) before, 3 and 12 months after treatment with high dose penicillin.

Results showed that there was no significant difference in blood CXCL13 content among 3 groups ($P > .05$); CSF CXCL13 content in neurosyphilis patients was significantly higher than in other 2 groups ($P < .001$), and positively related to leucocyte count, protein concentration, and IgG index. IL-6 and IL-10 contents of the serum and CSF in neurosyphilis patients were markedly higher than in other 2 groups ($P < .05$ or .01), but IL-2, IL-12, and IFN-γ of the serum and CSF were significantly lower than in other 2 groups ($P < .05$ or .01). The IL-6, IL-10, IL-2, IL-12, and IFN-γ contents of the serum and CSF were comparable between control group and syphilis group ($P > .05$). CSF CXCL13 content was positively related with IL-6 and IL-10 content, while negatively related to IL-12 content in neurosyphilis patients. CSF IL-6 content was negatively related with IL-12 content. In neurosyphilis patients, the CSF CXCL13 content reduced significantly at 3 and 12 months ($P < .001$), the CSF IL-2 and IL-12 contents increased significantly at 12 months, and CSF IL-6 contents reduced significantly at 12 months after treatment ($P < .05$ or .01).

It is concluded that neurosyphilis patients did not have normal immune function. CXCL13 and Th1/Th2 cytokines are involved in the immune response of neurosyphilis patients. CSF CXCL13 and Th1/Th2 cytokines contents may be used for the diagnosis and evaluation of therapeutic efficacy of neurosyphilis.

Abbreviations: CNS = central nervous system, CSF = cerebrospinal fluid, FTA-ABS = fluorescent treponemal antibody absorption, ICP = intracranial pressure, NND = noninflammatory disease, TRUST = Tolulized red unheated serum test, TP = *Treponema pallidum*, TPPA = *Treponema pallidum* particle agglutination, VDRL = venereal disease research laboratory.

Keywords: cerebrospinal fluid, CXCL13, cytokine, neurosyphilis

1. Introduction

Syphilis is a chronic and systemic sexually transmitted disease caused by *Treponema pallidum* (TP) infection. Neurosyphilis is a central nervous system (CNS) infectious disease with involvement of meninges, brain, and spinal cord due to the invasion of TP in the CNS. About 10% to 25% of untreated syphilis patients will develop neurosyphilis.[1] The clinical manifestations of neurosyphilis are diverse and usually nonspecific, which thus often cause mis-diagnosis and missed diagnosis. Thus, neurosyphilis is also named "great imitator."[2] Moreover, therapeutic efficacy is very poor for neurosyphilis at a late stage. However, regular treatment has the possibility to cure syphilis at early stage. Thus, clinicians should emphasize the diagnosis, treatment, and prognosis of neurosyphilis, and attempt to diagnose and treat syphilis at an early stage, aiming to improve its prognosis.

Immune response to TP plays important roles in the development and recovery of syphilis after TP infection. Chemokines and cytokines secreted by T cells possess specific immunoregulatory and killing activities, which play crucial roles in the anti-TP infection.[3,4] However, there are still controversies on the early diagnosis and prognosis assessment with cytokines. There is evidence showing that the cerebrospinal fluid (CSF) IFN-γ content increases in asymptomatic patients with neurosyphilis,[5] and CXCL13 content is helpful for the diagnosis of neurosyphilis in HIV-negative patients.[6,7] To investigate the cellular immunity of HIV-negative neurosyphilis, this study was conducted to detect the CXCL13 and Th1/Th2 cytokines contents of the serum and CSF in HIV-negative patients with neurosyphilis before and after treatment, aiming to investigate the roles of these factors in the immune response and

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pathogenesis of neurosyphilis, which may provide evidence for the early diagnosis and evaluation of therapeutic efficacy of neurosyphilis.

2. Materials and methods

2.1. Ethics

All procedures were conducted in accordance with the ethics standards of the responsible committee on human experimentation and with the Helsinki Declaration as revised in 1983. And this study has been approved by the Ethics Committee of Hangzhou third Hospital.

2.2. Clinical characteristics

A total of 47 patients were admitted to the Department of Neurology and received treatment for neurosyphilis between July 2011 and December 2014. There were 27 males and 20 females with the mean age of 41.7 ± 12.9 years (range: 23–72 years). The mean course of disease was 2.4 ± 1.3 years (range: 0.5–6.1 years). All the patients met the diagnostic criteria for neurosyphilis[8]: presence of clinical symptoms and signs of neurosyphilis and causes except for syphilis were not identified; serological tests of non-TP antigen and TP antigen showed both positive; CSF examination: leucocyte count was $\geq 10 \times 10^6$/L and protein concentration was >50 mg/dL, and causes except for neurosyphilis were not identified; venereal disease research laboratory (VDRL) test or fluorescent treponemal antibody absorption (FTA-ABS) test showed positive. On the basis of clinical manifestations, neurosyphilis patients were divided into symptomatic and asymptomatic neurosyphilis patients. On the basis of involved tissue, neurosyphilis patients were divided into early and late neurosyphilis patients.

In addition, 36 outpatients with syphilis (without CNS involvement) were recruited from the Department of Dermatological and Sexually Transmitted Diseases. There were 15 males and 21 females. The mean age was 36.7 ± 12.9 years (range: 18–62 years). The mean course of disease was 1.6 ± 1.1 years (range: 0.04~5.0 years). All the patients met the diagnostic criteria for syphilis[8]: patients had a history of illegitimate sexual intercourse; patients had clinical symptoms and signs of syphilis; and Tolulized red unheated serum test (TRUST) and TP particle agglutination (TPPA) tests showed positive.

In the control group, 23 patients with neurological noninflammatory disease (NND) were recruited from our hospital. Of them, 14 were diagnosed with migraine, 2 with Alzheimer disease, and 7 with epilepsy. There were 10 males and 13 females. The mean age was 38.9 ± 12.6 years. TRUST and TPPA tests showed negative.

Other infectious diseases (HIV) and autoimmune diseases were excluded from above patients by other tests.

2.3. Serological tests

Ten milliliters of venous blood was collected into a vacuum tube and then centrifuged at 3000 r/min for 5 minutes. The serum was collected, transferred into an Eppendorf tube, and stored at −20°C. TRUST kit was purchased from Shanghai Rongsheng Biotech Co., Ltd (Shanghai, China), and TPPA kit was from Fujirebio Inc (Tokyo, Japan). IL-2, IL-12, IFN-γ, IL-6, and IL-10 were detected with enzyme-linked immunosorbent assay and kits were purchased from Shanghai Qiming Biotech Co., Ltd. (Shanghai, China). All the cytokines were detected according to the manufacturer's instructions and absorption was measured at 450 nm. Cytokine content was calculated according to the standard curve. Human CXCL13/BLC/BCA-1 Quantikine ELISA Kit (R&D Systems Europe Ltd., Europe) was employed for the detection of CXCL13 content according to the manufacturer's instructions.

2.4. Detection of CSF

CSF was collected after informed consent was obtained from each patient. After lumbar puncture (lumbar puncture was also performed at 3 and 12 months after treatment in neurosyphilis patients), 7 mL of CSF was collected into a sterilized tube for routine CSF test, CSF biochemistry IgG index measurement, TRUST test, TPPA test, FTA-ABS test, VDRL test, and detection of IL-2, IL-12, IFN-γ, IL-6, IL-10, and CXCL13 contents. Detection of IL-2, IL-12, IFN-γ, IL-6, IL-10, and CXCL13 in CSF was done with the same method used for the serum cytokine contents. VDRL kit (BD Biosciences, New Jersey, USA) and FTA-ABS kit (Trinity Biotech plc, Dublin, Ireland) were used, and measurements were performed according to the manufacturer's instructions.

2.5. Treatments

For neurosyphilis patients, Penicillin sodium was intravenously infused at 2.4×10^7 U/day (0.4×10^7 U/4 h) for consecutive 14 days, and thereafter, benzathine penicillin G was intramuscularly injected at 2.4×10^6 U/week for consecutive 3 weeks.

2.6. Statistical analysis

SPSS software (version 17.0; SPSS Inc, Chicago, IL) was adopted. Kolmogorov–Smirnov was used to test normality. Measurement data with normality distribution were shown as $\bar{x} \pm s$. *t* test was used to compare between 2 groups. Variance analysis was used to compare among multiple groups. Chi-square test was used to analyze enumeration data. Data with abnormal distribution were described as median [M(Q1-Q3)]. Mann–Whitney *U* test was adopted to compare these data between 2 groups. Spearman correlation analysis was adopted to analyze correlation. $P < .05$ in 2-sided test was considered statistically significant.

3. Results

3.1. General characteristics

Of 47 neurosyphilis patients, 26 patients were symptomatic and 21 patients had no symptoms. Among them, 33 cases were early neurosyphilis patients, and 14 cases were late neurosyphilis patients. There were no significant differences in the age and gender among neurosyphilis group, syphilis group, and control group ($P > .05$). The course of disease in neurosyphilis was significantly longer than in syphilis group ($P < .01$).

3.2. Serum cytokine contents in different groups

The serum IL-6 and IL-10 contents in neurosyphilis group were significantly higher than in syphilis group and control group ($P < .05$ or .01), but the serum IL-2, IL-12, and IFN-γ contents in neurosyphilis group were markedly lower than in syphilis group and control group ($P < .05$ or .01). Moreover, the serum IL-6, IL-10, IL-2, IL-12, and IFN-γ contents were comparable between syphilis group and control group ($P > .05$). There was no

Table 1

Serum and CSF contents of IL-2, IL-12, IFN-γ, IL-6, IL-10, and CXCL13 in different groups [M(Q1-Q3)] (pg/mL).

Group	n	IL-2		IL-12		IFN-r		IL-6		IL-10		CXCL13	
		Serum	CSF	Serum	CSF	Serum	CSF	Serum	CSF	Serum	CSF	Serum	CSF
Neurosyphilis	47												
Baseline		123 (114–147)	136 (121–158)	31.7 (22.1–38.8)	30.2 (22.4–38.8)	3.8 (3.0–4.3)	3.8 (3.0–4.3)	5.3 (3.6–7.6)	5.4 (4.3–7.8)	2.43 (2–3.5)	3.2 (2.1–4.0)	128.8 (99.3–156.2)	121.5 (87.3–165.2)
3 mo		124 (109–154)	144 (134–165)	32.5 (24–46.3)	32.6 (21.8–42.6)*	3.8 (2.8–5.3)	3.8 (2.7–4.9)	5.3 (3.3–6.3)	4.6 (3.5–6.7)	2.5 (1.9–3.2)	2.8 (1.9–3.8)	110.5 (102.1–143.7)	11.2 (8.4–17.9)‡
12 mo		124 (109–154)	154 (133–169)†	33.7 (24.1–47.6)	42.3 (32.5–54.6)*	3.7 (2.6–5.2)	4.1 (3.4–5.2)*	5.1 (3.6–6.3)	4.6 (3.3–6.3)†	2.3 (1.6–3.1)	2.5 (1.9–3.2)	111.4 (85.6–147.5)	5.7 (3.3–9.5)‡,§
Syphilis	36	143 (120.3–162.8)†	147 (136–165.8)†	38.7 (30.2–60.1)†	38.7 (32.2–56.3)†	4.0 (3.2–5.4)	4.2 (3.3–5.6)*	4.2 (2.6–7.0)	4.6 (2.7–7.1)	2.2 (2.0–2.9)†	2.3 (2.0–3.35)†	112.4 (89.4–166.6)	4.9 (3.5–6.8)‡
Control	23	144 (124–174)†	154 (144–166)†	38.8 (31.3–60.7)*	38.8 (33.6–56.8)†	4.0 (3.5–6.3)*	4.5 (3.4–5.6)†	3.7 (2.7–6.1)†	4.1 (2.6–5.9)†	2.0 (1.7–2.4)*	1.9 (1.5–3.4)*	110.5 (100–133.2)	5.1 (2.8–6.4)‡

CSF = cerebrospinal fluid.
* $P < .01$.
† $P < .05$.
‡ $P < .001$ versus baseline in neurosyphilis group.
§ $P < .01$ versus 3 mo in neurosyphilis group.

significant difference in serum CXCl13 content among 3 groups ($P > .05$) (Table 1).

3.3. CSF cytokines in different groups

The intracranial pressure (ICP) was similar among 3 groups ($P > .05$). The protein concentration, leukocyte count, and IgG index in neurosyphilis group were significantly higher than in syphilis group and control group ($P < .001$), but there were no marked differences in them between syphilis group and control group ($P > .05$) (Table 2).

In neurosyphilis group, the CSF contents of IL-6, IL-10, and CXCL13 were significantly higher than in syphilis group and control group ($P < .05$ or .01), but those of IL-2, IL-12, and IFN-γ were markedly lower than in syphilis group and control group ($P < .05$ or .01). The CSF contents of IL-6, IL-10, IL-2, IL-12, IFN-γ, and CXCL13 were comparable between control group and syphilis group ($P > .05$) (Table 1).

3.4. Serum and CSF cytokines after treatment in neurosyphilis patients

At 3 and 12 months after treatment, the serum contents of CXCL13, IL-2, IL-12, IFN-γ, IL-6, and IL-10 remained unchanged ($P > .05$), but CSF CXCL13 content reduced significantly ($P < .001$), CSF contents of IL-2, IL-12, and IFN-γ increased gradually, CSF IL-6 and IL-10 contents reduced progressive after treatment. Among them, IL-2, IL-12, and IL-6 contents at 12 months after treatment showed significant difference from those before treatment ($P < .05$ or .01) (Table 1). The ICP remained unchanged, but CSF leukocyte count, protein concentration, and IgG index reduced dramatically after treatment ($P < .05$ or .001) (Table 2).

3.5. Correlation analysis of CSF parameters

In neurosyphilis patients, there was no significant correlation between serum and CSF CXCL13 contents ($P > .05$). Then, the correlation of CSF CXCL13 with other CSF parameters was further analyzed. Results showed that CSF CXCL13 content was positively related to CSF leukocyte count, protein concentration, and IgG index ($r = 0.3245$, $P = .0261$; $r = 0.5435$, $P < .0001$; $r = 0.7500$, $P < .0001$) (Figs. 1–3). CXCL13 content in CSF was positively related with IL-6 and IL-10 ($r = 0.3326$, $P = .0223$; $r = 0.3816$, $P = .0081$), and negatively related with IL-12 ($r = -0.3032$, $P = .0383$). IL-6 content in CSF was negatively related to IL-12 ($r = -0.3228$, $P = .0269$). However, there was not any correlation among various serum cytokine contents in patients with neurosyphilis (Tables 3 and 4).

4. Discussion

In recent years, the prevalence of syphilis is increasing and the incidence of neurosyphilis is also increasing over year. There is evidence showing that about 10% to 25% of untreated syphilis patients will develop neurosyphilis,[1] and some clinicians propose that neurosyphilis will be present at an early stage of syphilis.[9] The clinical characteristics of neurosyphilis are diverse, leading to the mis-diagnosis and missed diagnosis of neurosyphilis. Moreover, the involvement of CNS may cause functional impairment at early stage or irreversible organic dysfunction at late stage, or even cause death. Thus, clinicians should emphasize the diagnosis of neurosyphilis. The clinical

Table 2

CSF parameters in 3 groups.

Group	n	ICP, mmH_2O	Protein concentration, mg/dL	Leukocyte count, $\times 10^6$/L	IgG index
Neurosyphilis	47				
Baseline		150.3±31.3	39.3 (25.4–52.0)	13 (7–22)	1.3 (0.9–1.8)
3 mo		142.0±19.6	38.7 (25.0–47.0)	4 (2–11)[‡]	0.7 (0.5–1.2)[‡]
12 mo		145.3±19.2	37.9 (32.0–43.1)[†]	3 (2–6)[‡,§]	0.6 (0.5–0.8)[‡,§]
Syphilis	36	142.8±18.9	20.9 (15.3–38.7)[*]	2 (1–5)[‡]	0.6 (0.5–0.7)[‡]
Control	23	139.4±21.3	28.5 (17.9–38.2)[*]	2 (1–3)[‡]	0.5 (0.4–0.6)[‡]

CSF = cerebrospinal fluid.
[*] $P < .01$.
[†] $P < .05$.
[‡] $P < .001$ versus baseline in neurosyphilis group.
[§] $P < .05$ versus 3 mo in neurosyphilis group;

manifestations of neurosyphilis are diverse, thus the diagnosis of neurosyphilis should be based on clinical manifestations, TP serological test, and CSF examination, and a single examination is not enough to confirm its diagnosis.[10,11] In this study, serum TRUST test and TPPA test showed positive, CSF leukocyte count and protein concentration increased to different extents, and TPPA and VDRL tests of the CSF showed positive in these patients, which met the diagnostic criteria for neurosyphilis. Moreover, 55% of patients were symptomatic.

The pathogenesis of neurosyphilis is still poorly understood, and immune response (especially the cellular immunity) has been a hot topic in the studies about the pathogenesis of neurosyphilis. It has been proposed that the nerve invasiveness, host cell immunity, and concomitant HIV infection are related to the occurrence of neurosyphilis. After TP infection, the immune response to TP plays important roles in the occurrence, development, and recovery of neurosyphilis, especially the T lymphocyte response to TP in the CNS.[12] In the human immune system, T lymphocytes are mainly responsible for the killing of TP, protecting the human body against infection.[13] Currently, studies reveal that the immune cells related response to TP has involvement of Th1 and Th1 cytokines. Th1 cytokines include IL-2, IL-12, and IFN-γ, and IL-6 and IL-10 belong to Th2 cytokines. T lymphocytes can secrete a variety of cytokines, exerting immunoregulatory and killing activities, which play important roles in the anti-TP infection.[3] In a rabbit model, Fitzgerald [14] proposed the Th1 shift to Th2 hypothesis to explain the progression of syphilis. They speculate that TP after entering the human body may induce the cellular response (Th1 like response), which may kill a large amount of TP, but thereafter mononuclear macrophages and lymphocytes are activated to produce a large amount of prostaglandins that inhibit Th1 response and selectively activate Th2 response. The production of Th2 cytokines further inhibit the Th1 response, leading to the deterioration of Th1 inhibition.[14,15] Thereafter, some studies confirm this hypothesis.[16,17] In the present study, the Th1 cytokines (IL-2, IL-12, and IFN-γ) of the serum and CSF in neurosyphilis patients were significantly lower than in syphilis group and control group, but Th2 cytokines (IL-6 and IL-10) increased as compared with latter 2 groups. This indicates that the Th2 cytokines are dominant and there is Th1/Th2 immune response imbalance in neurosyphilis patients, which inhibits the clearance of TP and leads to the long-term tolerance to TP and the latency of TP. According to the symptoms, neurosyphilis is classified as asymptomatic and symptomatic [mesenchymal (meningovascular), cerebral parenchymal (general paresis and tabes dorsalis), and gumma]. Meanwhile, it can also be divided into early stage and late stage[18] based on the involved tissues. Early neurosyphilis refers to that TP only involves meninges and related vessels, while late neurosyphilis involves brain parenchyma and spinal cord. In this study, 33 early neurosyphilis and 14 late neurosyphilis were included. Compared with the late

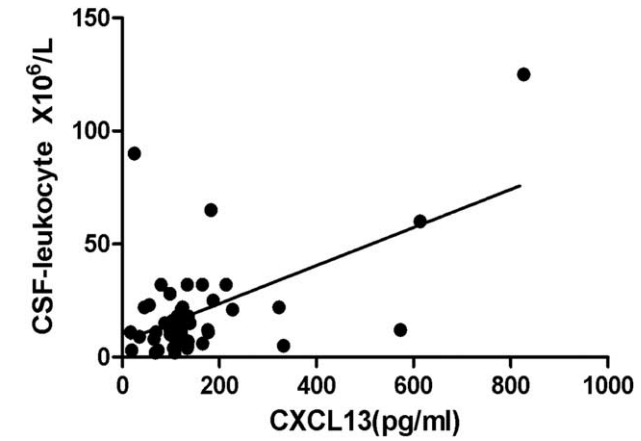

Figure 1. Correlation between CSF CXCL13 and CSF leukocyte count. CSF CXCL13 content was positively related to CSF leukocyte count (Spearman correlation analysis, $r = 0.3245$, $P = .0261$).

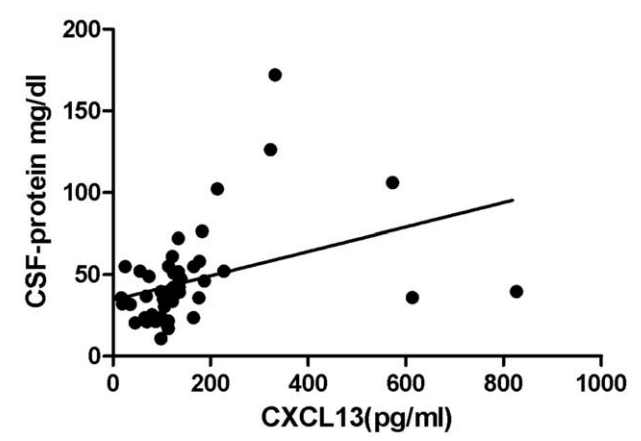

Figure 2. Correlation between CSF CXCL13 and CSF protein concentration. CSF CXCL13 content was positively related to protein concentration (Spearman correlation analysis, $r = 0.5435$, $P < .0001$).

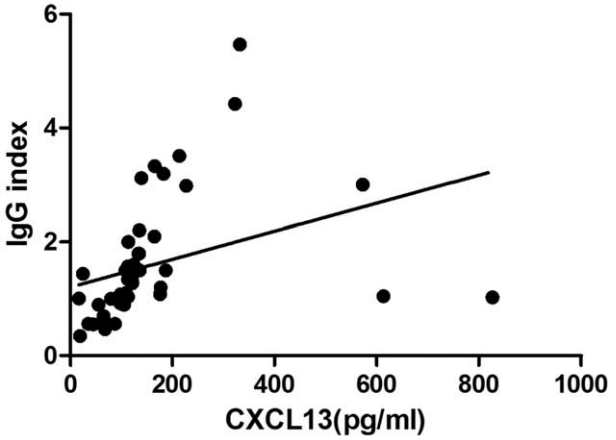

Figure 3. Correlation between CSF CXCL13 and CSF IgG index. CSF CXCL13 content was positively related to IgG index (Spearman correlation analysis, $r = 0.7500$, $P < .0001$).

neurosyphilis patients, IL-2, IL-12, IFN-r, IL-6, IL-10, and CXCL13 concentrations in serum and CSF showed no significant difference in the early neurosyphilis patients ($P > .05$). This might be influenced by the similar course of disease between both groups (2.64 ± 1.8 vs 2.36 ± 1.1, $t = 0.10$, $P = .9226$) as well was the small sample sizes.

Chemokines are a group of cytokines secreted by some cells. CXCL13 is a member of CXC chemokine family, which is mainly secreted by secondary lymphoid tissue, lymph nodes, and dendritic cells, reflecting the immune response after CNS infection.[19] Several studies[20–22] have revealed that CSF CXCL13 concentration may aid the diagnosis of symptomatic/asymptomatic neurosyphilis, and CSF CXCL13 content reduces after effective treatment. These indicate that CSF CXCL13 may be used to evaluate the therapeutic efficacy. In the present study, results also indicated that CSF CXCL13 content in neurosyphilis patients was significantly higher than in syphilis group and control group, and CSF CXCL13 content was positively related to leukocyte count, protein concentration, and IgG index, but there was no significant correlation between serum and CSF CXCL13. These suggest that the increased CXCL13 in the CSF patients might be synthesized in CNS cells after TP stimulation, but not from the peripheral blood. This study showed that CXCL13 concentration in CSF was related to serum/CSF TRUST titer but without statistical significance ($r = 0.1592$, $P = .2851$; $r = 0.0432$, $P = .7733$). Besides, increased serum/CSF TRUST titer, which is $\geq$1:16, is consistent with previous studies.[23,24]

There is a wide correlation among various cytokines. Different cytokines can activate with, cooperate with, inhibit, or antagonize receptors on membrane surface and soluble receptors, which is very complex and called cytokine network.[25] Sporn and Roberts [26] and Kawade[27] defined cytokines and cytokine network as communication language among cells, providing a new thinking for cytoinformatics including cytokine network. Effect of cytokine network on neurosyphilis process is still unknown. This study showed that CXCL13 content was positively related to IL-6 and IL-10, and IL-6 was negatively related to IL-12, indicating that there was interaction and regulation among chemokines and cytokines in neurosyphilis patients. Previous studies[3,5–7,28] only tested expression of a few cytokines or chemokines, which is difficult to reveal correlation between cytokines or between cytokines and chemokines. This study tested 5 cytokines and chemotactic factor CXCL13 content in serum and CSF in neurosyphilis patients and analyzed the correlation among them. Results showed that there was correlation among cytokines and chemokines in neurosyphilis patients. These cytokines influenced mutually with each other, instead of simple regulation or antagonism. However, the mechanism needs further study. Thus, it is essential to find the same upstream substance regulating cytokines and chemokines, which can clarify immune mechanism better.

Table 3

Correlation analysis of CSF contents of IL-2, IL-12, IFN-γ, IL-6, IL-10, and CXCL13 in neurosyphilis patients.

	IL-2		IL-12		IFN-r		IL-6		IL-10		CXCL13	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
IL-2	1	—										
IL-12	−0.1941	.1912	1	—								
IFN-r	−0.0939	.5302	0.1388	.3523	1	—						
IL-6	−0.2205	.1364	−0.3228	.0269	0.0003	.9983	1	—				
IL-10	0.2055	.1657	−0.1641	.2703	0.0718	.6311	0.1352	.3650	1	—		
CXCL13	0.1477	.3216	−0.3032	.0383	−0.1333	.3717	0.3326	.0223	0.3816	.0081	1	—

CSF = cerebrospinal fluid.

Table 4

Correlation analysis of serum contents of IL-2, IL-12, IFN-γ, IL-6, IL-10, and CXCL13 in neurosyphilis patients.

	IL-2		IL-12		IFN-r		IL-6		IL-10		CXCL13	
	r value	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
IL-2	1	—										
IL-12	−0.0792	.5968	1	—								
IFN-r	0.2573	.0808	−0.1453	.3298	1	—						
IL-6	0.0349	.8161	−0.0678	.6506	0.1263	.3975	1	—				
IL-10	−0.1613	.2786	−0.0376	.8020	−0.0255	.8651	0.0934	.5326	1	—		
CXCL13	0.1119	.4540	−0.0577	.7002	−0.2444	.0980	0.0923	.5371	−0.1951	.1888	1	—

Penicillin has been used in the treatment of syphilis since 1943, and has become a treatment of choice for syphilis at different stages.[29] Although great progress has been achieved in the development of antibiotics and some studies also report the effectiveness of macrolides and tetracyclines in the treatment of syphilis, penicillin is still an indispensable treatment of choice for syphilis. Tattevin et al[30] and Serragui et al[31] found that high-dose penicillin was required for the effective treatment of neurosyphilis, and penicillin at a routine dose may not be effective to kill TP due to the low CSF concentration. In the present study, the CSF leukocyte count, IgG index, and protein concentration reduced significantly after 2-week treatment in 47 patients with neurosyhilis, suggesting the effectiveness of penicillin. Moreover, after regular treatment, CSF CXCL13 content reduced gradually, IL-2 and IL-12 content increased, IL-6 content decreased, and the Th1/Th2 immune response imbalance was also improved. Thus, we speculate that CSF CXCL13 and Th1/Th2 cytokines may provide evidence for the diagnosis and evaluation of therapeutic efficacy of neurosyphilis. It can also indicate that the cytokine network is a dynamic equilibrium network in neurosyphilis patients. In healthy people, various cytokines and chemokines are under an equilibrium state. The cytokine network will change and the immune response will lean to Th2, when body was infected with TP. The cytokine network will also recover, when the infection is under control. The process of dynamic equilibrium is also the process of self-stabilization. Results of regulation make body maintain the dynamic equilibrium, function well, while loss of regulation will break the dynamic equilibrium, leading to a pathological state.

Of course, there were several limitations in this study. The sample size was small and follow-up period was relatively shorter. In addition, the correlations of serum and/or CSF cytokines with clinical manifestations, disease severity, and outcomes were not further assessed. Moreover, whether CSF CXCL13 and Th1/Th2 cytokines are more sensitive than leukocyte count and protein concentration in the diagnosis of neurosyphilis was not further investigated in this study. Thus, more studies with large sample size are needed to confirm our findings in patients with syphilis at different stages.

Taken together, there is dysfunction of cellular immunity in neurosyphilis patients, and CXCL13 and Th1/Th2 cytokines are involved in the immune response in case of neurosyphilis. CSF CXCL13 and Th1/Th2 cytokines may be used for the diagnosis and evaluation of therapeutic efficacy of neurosyphilis. More controlled studies are warranted to elucidate the change in CXCL13 and Th1/Th2 cytokines after treatment as well as at different stages in patients with syphilis.

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