

## Original Article

# Treponemal antibody in CSF and cellular immunity in peripheral blood of syphilitic patients with persisting positive rapid plasma regain

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Received January 25, 2015; Accepted March 22, 2015; Epub May 1, 2015; Published May 15, 2015

**Abstract:** The ratio of patients with RPR constant positive more than 2 years despite receiving standard syphilis treatment has been reported to be 11.54%~31.3%. The current interpretations on this phenomenon are cellular immune function restrained and the existence of neurosyphilis or asymptomatic neurosyphilis. We conducted this study to detect the treponemal antibody in cerebrospinal fluid (CSF) and lymphocyte subsets in peripheral blood of syphilis patients with persisting RPR positive more than 2 years without neurologic signs, and then explore their relationship. In this study, Treponemal antibody in CSF of 46 syphilitic with HIV negative were measured by syphilitic serum test and compared with that of 5 neurosyphilis. Lymphocyte subsets were measured by flow cytometry (FCM) and compared with that of 30 healthy controls. We observed that treponemal antibody in CSF was detected not only in 12 cases (25.21%) of 46 treated patients, but also in 5 neurosyphilis. The ratio of lymphocyte subsets revealed that CD3<sup>+</sup>, CD4<sup>+</sup> T cells and natural killer (NK) cells showed no significant differences between the patient and healthy controls ( $P > 0.05$ ), while CD8<sup>+</sup> T cells in patients were significant higher than that in healthy controls ( $P < 0.001$ ). Lymphocyte subsets showed no significant differences between the patients with treponemal antibody positive and negative in CSF ( $P > 0.05$ ). In conclusion, the treponemal antibody in CSF of treated patients suggests that part of them were asymptomatic neurosyphilis and with cellular immunodeficiency. And there is no significant relationship between asymptomatic neurosyphilis and cellular immunodeficiency in peripheral blood.

**Keywords:** Syphilis, neurosyphilis, cerebrospinal fluid (CSF), T-lymphocyte subsets, natural killer cell, RPR

## Introduction

Syphilis can almost invade every organ or system of the body, and even lead to multiple organ damage. Over the past decade the incidence of syphilis increased significantly, as of the end of 2013, the incidence of syphilis in our country is about 29.16/100 000 [1]. In China, the standard treatment for early or late syphilis consists of benzathine penicillin 2.4 MU administered as intramuscular injections every week for 3 weeks. Although it is generally believed that after several courses of conventional treatment, the treponemal antibody in peripheral blood of syphilitic patients will become negative within two years (excluding the serofast status), however, some of these patients proved to be performed persisting RPR positive actually. Some scholars [2, 3] demonstrated the ratio of patients with RPR constant positive

more than 2 years despite receiving standard syphilis treatment was high up to 11.54%~31.3%. On this phenomenon, the current interpretation is cellular immune function restrained [4, 5], which decreases the ability of the body to remove the treponema pallidum. But some scientists believe it is because part of syphilis patients with neurosyphilis or asymptomatic neurosyphilis [6, 7]. In order to explore the incidence of the asymptomatic neurosyphilis whose RPR persisting positive after several courses of conventional treatment and its relationship with cellular immunity, we detected the syphilis antibody in cerebrospinal fluid (CSF) of 46 cases of asymptomatic neurosyphilis with RPR constant positive more than 2 years after several courses of therapy and 5 cases of untreated neurosyphilis by RPR, VDRL, TPHA and FTA-Abs test, and detected the lymphocyte subsets and NK cells in peripheral blood of

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these patients by flow cytometry at the same time.

### Materials and methods

#### *Reagents and sources*

Major reagents included the following: reagents for *Treponema pallidum* polymerase chain reaction (TP-PCR, Sun Yat-son Gene Diagnosis Center, China); reagents for rapid plasma regain (RPR, Hi-Tech Industrial Development Zone, Urumqi, the new company); reagents for Venereal disease research laboratory (VDRL, Omega, UK); reagents for *Treponema pallidum* haemagglutination (TPHA, Fujitsu Ltd. Japan); reagents for Fluorescent Treponemal Antibody Absorption Test (FTA-Abs, Military Medical Sciences Center, China); monoclonal antibody kit (Beckman Coulter, US); Flow cytometry (Beckman Coulter, US).

#### *Study participants*

As the patient group, 46 cases patients with persisting RPR positive more than two years after several conventional antisyphilitic treatments were chosen from the outpatient of our hospital (*Guangzhou Institute of Dermatology and Venerology*), comprising 20 male and 26 female patients. The average of the patients was 29.67 years, ranging from 24 to 33 years. All the samples were positive in RPR (titers from 1:2 to 1:8) and TPHA and IgM test, and negative in AIDS Initial Screening Test. And none of them has the clinical manifestations of the nerve damage. The control groups were consisted by the positive control group and the healthy control group. As the positive control group, we recruited 5 untreated latent syphilis patients without neurological damage manifested from other hospitals' neurology, which were diagnosed with neurosyphilis by clinical manifestations, MRI and serum RPR (titers from 1:16 to 1:64) and TPHA test, while their blood HIV screening tests were negative. At the same time we invited 30 healthy donors as the healthy control group, including 16 males and 14 females, aged 18 to 42 years, with an average age of 29.76. All of these groups were negative in Peripheral Blood Circulation HIV screening, RPR and TPHA test.

After obtaining written informed consent, we interviewed each participant for a detailed me-

dical history and performed a routine neurologic examination for assessment of cranial nerve function, motor function, sensation, reflexes, coordination, and gait. Venous blood and CSF were collected from all members for syphilis testing and evaluation per standard clinic practice at the hospital in Guangzhou. All the participants also underwent HIV antibody testing. This study was approved by the ethics committee of the Guangzhou Institute of Dermatology.

#### *Specimen collection and experimental procedure*

After routine disinfection, we collected cerebrospinal fluid 3~5 mL into sterile tube by lumbar puncture and sent it to inspection immediately. The detection of CSF samples refers to the manual of each reagent. Routine testing of CSF was conducted to examine the inflammatory responses in the CNS, including the CSF white blood cell (WBC) count, red blood cell (RBC) count, glucose and total protein. The CSF samples underwent testing with TP-PCR, RPR, VDRL, TPHA, and FTA-Abs for neurosyphilis diagnosis, using the same procedures applied to serum specimens. Furthermore, syphilis dark field examination was used to detect *treponema pallidum* in CSF.

Blood samples were also collected from individuals at the same time and tested for syphilis serology on same day. Draw peripheral blood of the subjects into two tubes, each containing 3~5 mL, one tube using heparin anticoagulation, the other without anticoagulant, send them to inspection immediately. The serum without anticoagulant were used to RPR and TPHA testing, while peripheral blood with heparin anticoagulation were used to detect peripheral lymphocyte. The proportion of T lymphocyte subsets and NK cells were detected after separating lymphocytes conventionally. All the methods and operation procedures of the experiment refer to the manual for the reagents and instruments.

#### *Statistical analysis*

The Statistical Package for the Social Sciences for Windows (SPSS, version 13.0; Chicago, IL) was used for statistical analysis. Descriptive statistics were used to calculate the mean and standard deviation (SD). The chi-square test

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**Table 1.** Test results of 12 syphilis patients with positive treponemal antibodies in CSF

Number	CSF-FTA-Abs	CSF-TPHA
1	-	+
2	-	+
3	-	+
4	-	+
5	+	+
6	-	+
7	+	+
8	-	+
9	+	+
10	+	+
11	+	+
12	+	-
Total	6	11

CSF-TPHA, Treponema pallidum haemagglutination test on cerebrospinal fluids. CSF-FTA-Abs, Fluorescent Treponemal Antibody Absorption Test on cerebrospinal fluids.

was performed to compare the syphilis antibody in CSF, and the rest were compared by the *t*-test. Differences were considered to be statistically significant at two-sided *P* values of < 0.05.

### Result

#### *Cerebrospinal fluid detection*

We collected the CSF samples of 46 asymptomatic neurosyphilis patients with persisting positive RPR and take them to inspection immediately. In routine CSF testing there were no red or white blood cells detected, while the total protein and glucose were increased in 28 cases (60.87%) and 14 cases (30.44%) respectively. No treponema pallidum was found in dark field microscope (DFM). All samples were negative in TP-PCR, VDRL, RPR test; while 12 cases (27.39%) of syphilis patients were proved treponemal antibodies exist in CSF, **Table 1**.

For the untreated patients with neurosyphilis, no red blood cells and white blood cells were found in CSF samples, and the protein and glucose were increased mild to moderate. In accord with the patients above, the CSF samples were negative in DFM either. What's more, the treponemal antibodies could be found in all samples, including VDRL-reactive in 2 cases, FTA-Abs-reactive in 5 cases and TPHA-reaction

in 4 cases, while the TP-PCR, RPR test showed negative result.

#### *Determination of peripheral blood lymphocyte subsets*

Compared with the patients and healthy control group, we observed the difference of the expressing of CD3<sup>+</sup>, CD4<sup>+</sup> T cells and NK cells was not significant (*P* > 0.05), while the expressing of CD8<sup>+</sup> T cell in patients was much higher than that in the healthy control group, which was statistically significant (*P* < 0.001) (**Table 2**). What's more, no significant difference was found in the detection of the peripheral blood lymphocytes between the patients whose cerebrospinal syphilis antibody was positive and the negative (*P* > 0.05), **Table 3**.

### Discussion

By detecting the peripheral blood samples of 46 cases patients with persisting RPR positive more than two years after several conventional antisyphilitic treatment, we found their RPR, TPHA and syphilis IgM test were all reactive. Owing to IgM is an infectious index of syphilis [8]; these patients were proved to be infectious rather than RPR serofast.

Some recent research on neurosyphilis showed [9, 10]: Although the specificity of CSF-VDRL test is very high, its sensitivity is quite low (10% to 89%), even the patients with neurosyphilis activity could be CSF-VDRL non-reactive. So if we just rely on the diagnosis criteria of neurosyphilis developed by CDC [11], a considerable part of neurosyphilis especially asymptomatic neurosyphilis will be misdiagnosis. In a recently publication, Noy M *et al* [12] also observed that although most of Treponema pallidum which invaded the central nervous system could be removed or controlled, there were still about a quarter of untreated syphilis patients developed into neurosyphilis.

Although the approach to the diagnosis of asymptomatic neurosyphilis is varied, we haven't found an efficiency method with high specificity and sensitivity so far. By testing the neurosyphilis syphilis antibodies in cerebrospinal fluid, Seung *et al* [13] revealed that compared to the FTA-Abs and TPHA, the sensitivity of VDRL is quite low, that is, neurosyphilis with CSF-VDRL negative result cannot be ruled out,

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**Table 2.** Detection of lymphocyte subsets in peripheral blood of syphilitic patients with persisting RPR positive ( $\bar{x} \pm s$ )%

Group	Total	CD3 <sup>+</sup>	CD4 <sup>+</sup>	CD8 <sup>+</sup>	NK
The patient group	46	71.00 ± 9.07	36.56 ± 8.40	29.35 ± 6.98	14.90 ± 8.51
The healthy group	30	69.98 ± 5.79	35.25 ± 5.16	25.08 ± 4.34	14.91 ± 4.87
t		0.817	1.062	4.206	0.008
P value		0.418	0.294	0.000*	0.994

\*Comparison of the CD8<sup>+</sup> T cell in the samples with the patient group and the healthy group (P < 0.001).

**Table 3.** Detection of lymphocyte subsets in peripheral blood of syphilis patients with CSF-antibody-positive and CSF-antibody-negative ( $\bar{x} \pm s$ )%

Group	Total	CD3 <sup>+</sup>	CD4 <sup>+</sup>	CD8 <sup>+</sup>	NK
CSF-antibody-positive	12	70.62 ± 8.18	37.03 ± 7.84	30.65 ± 7.39	16.41 ± 6.46
CSF-antibody-negative	34	71.14 ± 8.73	36.44 ± 8.68	28.38 ± 6.72	14.36 ± 9.05
t		0.182	0.220	0.506	0.700
P value		0.857	0.827	0.615	0.484

CSF-antibody-positive, The patients with syphilis antibody positive in CSF-antibody-negative. CSF-antibody-negative, The patients with syphilis antibody negative in CSF-antibody-negative.

while the positive can be considered neurosyphilis, and neurosyphilis with CSF-FTA-Abs or CSF-TPHA positive result also indicates neurosyphilis. Park *et al* [14] pointed out, as long as the FTA-Abs and TPHA syphilis antibody of the blood and CSF are both positive, the specificity and the sensitivity for the diagnosis of neurosyphilis were not less than 94% and 87% separately, the VDRL test could be avoided. We detected the syphilis antibody in the cerebrospinal fluid of 46 cases syphilitic patients with persisting RPR positive more than 2 years, and found that the VDRL, RPR test for all cases were non-reactive, while 12 cases (27.39%) of these patients proved to be exist syphilis antibodies, indicating that the detection rate of syphilis antibody in the cerebrospinal fluid of these patients is quite high. If followed the diagnostic criteria proposed by Park, our results also had 5 cases of this kind of syphilis whose syphilis antibody are both positive in CSF-FTA-Abs and CSF-TPHA test, the positive rate was 10.87%, which indicated that if we take the CSF-VDRL-reactive as the sole diagnostic criteria of asymptomatic neurosyphilis, some of these patient will be missed diagnosis. Thus we believe that detecting the syphilis antibody in cerebrospinal fluid by the TPHA and FTA-Abs test is more significance than the VDRL test in diagnosis of asymptomatic neurosyphilis.

García P *et al* [15] applied *Treponema pallidum* PCR (TP-PCR) to detect the CSF of neurosyphilis patients and the results showed that 5 cases

in 7 acute neurological syphilis and 2 cases in 16 asymptomatic neurosyphilis patients were positive, while 4 chronic nerve syphilis patients were all negative. A research by Chung *et al* [16] also reported that PCR of the CSF from syphilis patients showed positive reaction in 4 of 6 (25%) primary and secondary syphilis patients, in 2 of 7 (29%) early latent syphilis patients, and in 2 of 3 (67%) late latent syphilis patients. Among the CSF sample of patients with TP-PCR-reactive, 1/8 (13%) presented VDRL reactive, while 1/16 (6%) showed VDRL negative result. They considered although CSF-TP-PCR showed reactive in all stages of the syphilis, it just suggested the *treponema pallidum* had invaded the CNS on early infective stage, that is, *T. pallidum* in the CSF was not correlated with the results of other tests used in the WHO criteria, and its significance in the diagnosis of neurosyphilis should further be evaluated. To the fact that CSF-TP-PCR was non-relative in some neurosyphilis patients, Villanueva *et al* [17] hold the opinion that it may be because there is none or just a very tiny amounts of *treponema pallidum* in CSF. We used TP-PCR to detect the CSF of 46 syphilitic patients with persisting RPR positive more than 2 years after several courses of treatment and 5 untreated neurosyphilis patients, and all the results were negative.

In the human immune system, cellular immune system plays an important role to resist pathogenic spirochetes and protect the body from

syphilis infection. Our research showed, compared with the healthy people, the CD3<sup>+</sup>, CD4<sup>+</sup> T cells and NK cells in peripheral blood of the syphilitic patients with persisting RPR positive more than 2 years after several courses of treatment makes no significant difference, while the CD8<sup>+</sup> T cells of these patients was significantly higher, which indicated that RPR persisting positive despite several courses of treatment had some correlation with the degree of immunosuppression. Our results are consistent with the report of Cruz AR *et al* [18], in which they detected activated CD8<sup>+</sup> T cells in skin lesions of the primary and secondary syphilis syphilis by immunopathology. Since the cellular immune response of the syphilis is mainly mediated by cells in delayed-type hypersensitivity (DTH) reaction, and CD8<sup>+</sup> T cells can not only inhibit the body's immune response, but also restrain the production of the treponema pallidum immobilizing antibody, we assume the significantly increased of the CD8<sup>+</sup> T cells in peripheral blood causes the proliferation and spread of the *Treponema pallidum*. From this perspective, we believed that add some appropriate immunity regulator on the basis of conventional treatment of syphilis could obviously improve its curative effect.

Additionally, as we see in **Table 3**, no significant difference was found in peripheral blood detection of T lymphocyte subsets and NK cells between the CSF-reactive and CSF-negative syphilis, which indicated that there was no direct correlation between the syphilis patients with CSF-RPR positive result more than 2 years despite several courses of treatment and the degree of the cell immunosuppression.

### Acknowledgements

This work was supported by grants from the Guangzhou science and technology key projects Foundation (2012-Z-102-05). We sincerely appreciate the clinic staff who recruited patients and we thank all participants of this study for their cooperation. We also thank the First Affiliated Hospital of Sun Yat-Sen University for critical comments on the manuscript.

### Disclosure of conflict of interest

None.

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