

## Exploring Serologic Indicators for Laboratory Diagnosis of Asymptomatic Neurosyphilis

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### Abstract

**Background:** Diagnosis of asymptomatic neurosyphilis (NS) has been challenging. The gold standard for NS diagnosis relies on venereal disease research laboratory (VDRL) test of cerebrospinal fluids (CSF). The aim of this study was to examine criteria for lumbar puncture and CSF examinations in asymptomatic NS patients.

**Methods:** One hundred and twenty-three syphilis inpatients were recruited, including 24 NS and 99 non-NS cases. NS diagnosis was based on hospital discharge. Clinical data were collected. Serological tests included *Treponema pallidum* particle agglutination assay (TPPA), the toluidine red unheated serum test (TRUST), and concentrations of serum IgG, IgA, IgM and albumin. Parameters of CSF examinations included reactivity of VDRL test and TPPA, white blood cell counts (WBC), levels of CSF IgA, IgG and IgM, levels of CSF albumin, total protein and glucose.

**Results:** Neurologic manifestations were significantly associated with NS. The titres and reactivity of serum TRUST and TPPA, and the concentrations of serum IgG, IgA, IgM and albumin were not significantly different between NS and non-NS patients. In CSF, 95.8% NS patients were VDRL reactive, and all non-NS patients were VDRL non-reactive. Levels of CSF WBC, IgG, IgA, IgM, albumin and total protein were significantly higher in NS than non-NS patients. Levels of CSF glucose and chloride were not different between NS and non-NS patients. The indexes of IgG and IgM and the quotient of albumin were significantly higher in NS than in non-NS patients. Multiple logistic regression analysis revealed that among the CSF and non-CSF indicators only neurologic manifestations were significantly associated with NS.

**Conclusions:** In addition to CSF parameters neurologic manifestation was significantly associated with NS. Serologic parameters seem not sensitive laboratory indicators for diagnosis of NS.

**Keywords:** Neurosyphilis; Diagnosis; Serological tests; Cerebrospinal fluid examinations

### Abbreviations

CNS: Central Nervous System; CSF: Cerebrospinal Fluid; NS: Neurosyphilis; TPPA: *Treponema pallidum* Particle Agglutination Assay; TRUST: Toluidine Red Unheated Serum; VDRL: Venereal Disease Research Laboratory; WBC: White Blood Cell Count

### Introduction

The causative agent of syphilis, *Treponema pallidum* subspecies pallidum, can affect the central nervous system (CNS) during any stage of the disease resulting in neurosyphilis (NS). Diagnosis of NS continues to be a challenge in clinical practice [1]. Laboratory diagnosis of NS relies on lumbar punctures and examinations of the cerebrospinal fluids (CSF). A lumbar puncture is recommended for any one of the following conditions: 1) congenital syphilis, 2) presence of neurologic or ophthalmic manifestations, 3) tertiary syphilis, 4) failure to respond to treatment, or 5) certain HIV-infected patients [2]. In clinical practice, these criteria are not always followed or are not

provided in some countries, which result in unnecessary lumbar puncture procedures. In the Chinese guidelines for neurosyphilis diagnosis, there are no criteria for lumbar punctures [3]. As a result, in the Shanghai Dermatology Hospital (SDH), a syphilis patient with neurologic manifestations or serum TPPA titres of  $\geq 640$  is most likely subjected to CSF examinations. Lumbar puncture to obtain CSF is an invasive procedure and can cause severe complications, and should be kept at minimum [4]. The purpose of this study was to determine whether serologic testing results could be used as indicators to conduct lumbar puncture and CSF examinations in NS-suspected patients.

### Methods

One Hundred and twenty-three (123) hospitalized syphilis patients in SDH during the period from November 2011 to January 2012 were recruited in the study. The criteria for recruitment of study participants were a combination of treponemal serological reactions and first time visitors with one of the follows: 1) wishing to exclude NS, 2) having neurologic or ophthalmic manifestations which cannot be explained otherwise, or 3) having high titres of serum TPPA ( $\geq 640$ ). Clinical records such as admission and discharge diagnoses and demographic

data were retrieved. The patients were divided into NS and non-NS groups based on hospital discharge diagnosis.

All participants were subjected to blood and serologic tests and CSF examinations. Laboratory tests of blood and CSF samples were those previously described [4,5]. Serum samples were tested for antibody reactivity using TPPA (Fujirebio Diagnostics, Inc., Tokyo, Japan) and the toluidine red unheated serum test (TRUST, Shanghai Rongsheng Biotech Co., Ltd., Shanghai, China). Concentrations of serum IgG, IgA, IgM and albumin were determined using the biochemistry system TBA-120FR (Toshiba Medical Systems Corporation Co., Ltd., Beijing, China). HIV infection was examined using the Chemiluminescence CHEMCLIN CC 600 method (Beijing Chemclin Biotech Co., Ltd., Beijing, China). CSF samples were tested for reactivity of venereal disease research laboratory test (VDRL, Becton, Dickinson and company, MD, USA), TPPA titres, concentrations of IgG, IgA, IgM, albumin and total proteins by immunochemistry systems image 800 (Beckman Coulter, Inc., CA, USA). White blood cell (WBC) counts (XS-800i, Sysmex Corporation, Hyogo, Japan) and concentrations of chemicals (TBA-120FR, e.g. glucose and chloride) in CSF were determined. Pang's test on CSF was also performed. Red blood cell (RBC) counts were examined to determine the quality of the CSF samples [5].

Associations between categorical variables were analyzed by using X<sup>2</sup> test or Fisher's Exact test, and ANOVA was used to compare average levels of continuous measurements between NS and non-NS patients. Bivariate logistic regression was used to analyze the association of each serological variable or clinical manifestation with NS. Multiple logistic regression analysis with a backward elimination selection procedure was used to evaluate all of the variables for their independent contribution to the prediction of NS. P values of <0.05 were considered to be significant.

## Results

### Demographic data and clinical manifestations

Of the 123 hospitalized syphilis patients, twenty-four were diagnosed as having NS at hospital discharge and 99 were non-NS. Male patients accounted for 16.7% in the NS group and 60.6% in non-NS group. The age of the patients was 49.0 ± 10.6 (1 standard error, SE) for NS and 38.5 ± 13.7 for non-NS. More NS patients (62.5%, 15/24) had neurologic manifestations than non-NS patients (5.1%, 5/99) (P<0.001). A significantly lower proportion of NS patients (12.5%) had skin lesions than non-NS patients (38.4%) (P=0.012). One NS (1/24) and 3 non-NS cases (3/99) had HIV infection (P>0.05).

### Differences of serum parameters between NS and non-NS

The proportions of patients with serum TPPA titres of ≥640 were 100% for NS and 92.9% for non-NS (P=0.21). The proportion of serum TRUST positive was 100% in NS and 99% in non-NS. Distributions of TRUST titres showed no significant differences between NS and non-NS patients (data not shown). The concentrations of serum IgG, IgA, IgM and albumin also showed no significant differences between NS and non-NS (Table 1).

Logistic regression analysis on the extra-CSF parameters indicated that CNS manifestations were significantly associated with a higher risk of NS. Other clinical presentations and serologic parameters were not significantly associated with NS.

Parameters	NS (n=24) (Mean ± SE)	Non-NS (n=99) (Mean ± SE)	P value
Serum (g/L)			
IgG	11.48 ± 3.05	13.07 ± 3.57	0.047
IgA	2.37 ± 0.86	2.40 ± 0.84	0.869
IgM	1.23 ± 0.73	1.38 ± 0.65	0.326
Albumin	40.55 ± 4.22	41.52 ± 6.94	0.512
CSF (g/L)			
WBC (×10 <sup>6</sup> )	39.92 ± 54.93	2.74 ± 4.66	<0.001
IgG	0.160 ± 0.108	0.032 ± 0.026	<0.001
IgA	0.010 ± 0.006	0.003 ± 0.002	<0.001
IgM	0.009 ± 0.010	0.002 ± 0.006	<0.001
Albumin	0.375 ± 0.173	0.172 ± 0.084	<0.001
Total protein	0.533 ± 0.253	0.263 ± 0.107	<0.001
Glucose (mmol)	3.690 ± 2.123	3.352 ± 0.532	0.157
Chloride (mmol)	126.50 ± 2.83	125.76 ± 10.00	0.721
IgG index	1.646 ± 1.166	0.553 ± 0.102	<0.001
IgM index	0.960 ± 1.151	0.293 ± 0.852	0.002
Albumin quotient	9.509 ± 4.764	4.277 ± 1.985	<0.001
NS: Neurosyphilis; Non-NS: Non-neurosyphilis; SE: Standard error; CSF: Cerebrospinal fluids			
IgG index= (CSF IgG/CSF albumin)/ (Serum IgG/Serum albumin);			
IgM index= (CSF IgM/CSF albumin)/ (Serum IgM/Serum albumin);			
Albumin quotient= (CSF albumin/Serum albumin) × 1000			

Table 1: Parameters of laboratory tests in NS and non-NS patients.

### CSF parameters in NS and non-NS syphilis patients

VDRL was reactive in 95.8% (23/24) NS patients and non-reactive in all (99/99) non-NS patients. Levels of CSF parameters (e.g., CSF WBC counts, IgG, IgA, IgM, albumin and total protein) were significantly higher in NS than non-NS (P ≤ 0.002) (Table 1). Over 62% (15/24) NS patients and 15.2% (15/99) non-NS patients had elevated WBC counts (>5 × 10<sup>6</sup>/L) (P<0.001). Approximately 54% (13/24) NS patients and 5.1% (5/99) non-NS patients had elevated CSF total protein (>0.45 g/L) (P<0.001). Levels of CSF glucose and chloride concentrations were similar among NS and non-NS patients (P>0.05). The indexes of IgG and IgM and the quotient of albumin were significantly higher in NS than in non-NS patients (P ≤ 0.002). RBC counts of the CSF samples showed no significant differences between NS and non-NS (P=0.975, data not shown).

## Discussion

Laboratory diagnosis of neurosyphilis relies on examination of CSF samples. Guidelines in some countries have set criteria for patients with syphilis to have lumbar punctures and CSF examined while other countries have no guidelines for lumbar punctures [2,3,6,7]. Continuous uncertainty exists regarding which patients with syphilis

should undergo lumbar puncture for CSF examinations [6]. Our study indicated that the current practical procedures for selecting lumbar punctures remained to be further investigated. We found that the titres of serum TPPA and TRUST are insensitive indicators. Other laboratory parameters of serum tests such as concentrations of IgG, IgA and IgM are not associated with the likelihood of NS. Clinical manifestations are associated with NS; however, high levels of physicians' experience are required to make the judgements.

We found that in the patients with syphilis labeled with neurologic manifestations, only half of them were CSF VDRL reactive and had abnormal CSF WBC counts and elevated levels of CSF total proteins. Cerebrospinal fluid pleocytosis or elevated total protein in CSF is a standard marker of active inflammation within CNS, but it is nonspecific and low sensitive [8].

Marra et al. reported that neurosyphilis is significantly more common when the serum rapid plasma regain (RPR) titre is  $\geq 1:32$  [6,9]. We did analysis on serum TRUST titres and did not found significant differences between NS and non-NS patients. The discrepancy could be due to the difference of disease stages of the study subjects.

One of the main complications of late syphilis is the gummata formation, the sores that normally exist inside the body or on the skin of the patients. Cerebral syphilitic gummata are rare. Of the CSF tests, the VDRL test yields positive in 62%, the fluorescent treponemal antibody absorption test positive in 60%, and the *T. pallidum* hemagglutination assay positive in 83% [10].

Emerging evidence indicates that epigenetic modifiers might play a key role in the initiation and development of carcinomas [11]. For example, histone methyltransferase G9a is overexpressed in different types of carcinomas, including ovarian cancer, AML and lung cancer [12-14]. As epigenetically G9a is able to modify H3K9me2 at euchromatin regions and protect DNA methylation at imprinted control regions (ICRs) [15,16], the aberrant distribution of epigenetic markers might directly result in the initiation and development of carcinomas in humans. Therefore, understanding the levels of epigenetic modifiers, such as G9a, in the patients with late syphilis might facilitate the early diagnosis of gummata formation and treatment of this disease.

The CSF VDRL reactivity is the golden standard for NS diagnosis. Our criteria for NS are based on hospital discharge diagnosis. In the 24 NS cases, only one was CSF VDRL non-reactive, which had neurologic manifestations and skin lesions, abnormal levels of CSF IgG, total protein and albumin, CSF WBC =  $2 \times 10^6/L$ , titres of CSF TPPA >640. Therefore, the NS criteria used in our study are stringent as compared to the Canadian guidelines which include any one of the following: 1) CSF VDRL reactive, 2) corrected CSF WBC  $>5 \times 10^6/L$ , and/or 3) CSF protein  $>0.45$  g/L plus CSF FTA-ABS positive [2].

## Conclusions

Taking together, we concluded that: 1) CNS manifestations were associated with NS; 2) Titres of serum TRUST and high levels of TPPA titres ( $\geq 640$ ) were not associated with NS; 3) Concentrations of serum IgA, IgM, IgG and albumin were not associated with NS. Criteria for lumbar punctures and CSF examination for diagnosis of asymptomatic NS warrant further study.

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## Contributors

WG was the principal investigator who designed and led the study, participated in data collection, data analysis and preparation of the manuscript. YC participated in study design, led data analysis and preparation of the manuscript. LW and YY participated in data collection, conducted laboratory experiments. All authors helped plan the study, interpret data and critically revise drafts of the manuscript.

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