Serology Detection of *Treponema pallidum* in Cerebrospinal Fluid with Intracranial Infection


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Jakarta, 2018
Introduction

Syphilis

Chronic sexually transmitted disease
Has varying clinical manifestations and persist for a long time

Treponema pallidum subsp. pallidum (T. pallidum)
Spiral microorganism

Centers for Disease Control and Prevention (CDC)
Syphilis cases about 19.999 in 2014, increased 15.1% from 2013 in US.

Syphilis incidens with HIV infection 77 times higher among other population (CDC)

Syphilis is higher in area with high prevalence of HIV (East Europe, China)

For recent decades in US, there is men who have sex with men (MSM) population and often occurs in young Afro-American population, Hispanic, and now Europe and China.

World Health Organization. Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. 2013.p1-228

Centers for disease control and prevention. Sexually transmitted disease surveillance 2014. 2015.p1-149

Ho EL, Spudich SS. Neurosyphilis and the impact of HIV infection. Sexual health. 2015 Apr:1-7

Human Immunodeficiency Virus (HIV) epidemi

Syphilis is increased in HIV patients
Ulcer, no pain, chancre, lymphadenopathy

Asymptomatic, many years, just detected by serology test

6 weeks to 6 months

Infection

Primary (chancre)

Secondary (rash)

Latent syphilis (no signs of disease)

Tertiary

Incubation period 9–90 days (rerata 3 minggu)

(Meningovascular syphilis)

Early syphilis

rash, mucocutaneous lesion, and lymphadenopathy, could involved another organ

1/3 who doesn’t get therapy

Syphilis staging

Many years to a lifetime

Many years to a lifetime

Neurosifilis

Epidemiology unknown
Less report

Retrospective research

One of the complications of systemic syphilis with findings in CSF with or without obvious symptoms.

CDC report from 4 cities in America from 2002-2004 (Los Angeles, San Diego, Chicago, New York) → MSM HIV positive population with early syphilis, risk to get symptomatic neurosyphilis about 1,7%.³

Prospektif research → Silber et al in 1999 → neurosyphilis prevalence about 3,3% in suspected meningitis⁴

Blogg et al in Indonesia, subject in detention population in 2010, syphilis prevalence 5,1% for men and 8,5% for women.⁵

Ho EL, Spudich SS. Neurosyphilis and the impact of HIV infection. Sexual health. 2015 Apr:1-7
Clinical manifestations

- Early acute meningitis
- Cranial nerve
- Pain and numbness in extremities
- Memory loss and mental confusion

5 - 10 years → neuroinvasion → syphilis meningovascular

Symptoms:
- fever
- Headache
- Nausea / vomiting
- Head stiffness
- Visual disturbances
- Hearing loss
- Facial weakness
- Blurry
- Photophobia
- Uveitis

Diagnosis

No gold standard for neurosyphilis diagnosis

CDC

surveillance definition for epidemiology

Confirmed neurosyphilis

- Syphilis any stages
- Reactive CSF VDRL

Presumptive neurosyphilis

- Syphilis any stages
- Nonreactive CSF VDRL
- CSF pleositis/protein increased
- Clinical symptoms consistent with syphilis without alternative diagnosis

Ghanem KG. Neurosyphilis: a historical perspective and review. CNS Neuroscience & Therapeutics. 2010;16:e157-68
Pleositosis in CSF lymphocyte domination, cutoff for cell count \( \geq 5 \text{ cell/\(\mu\text{L}\).} \) But, cell count cutoff was not specific for HIV patients. Marra et al were using cell count cutoff 20 cell/\(\mu\text{L}\) in HIV patients.  

CSF protein was varying based on neurosyphilis clinical staging. In syphilis meningitis, 12% CSF protein \( \leq 45 \text{ mg/dL}, \) 34% in meningovascular syphilis, 25% in general paresis, and 47% in tabes dorsalis.  

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Ghanem KG. Neurosyphilis: a historical perspective and review. CNS Neuroscience & Therapeutics. 2010;16:e157-68

Antibody were produced as syphilis infection response, that used by clinician for diagnosis.

Syphilis serology first developed in early 20th century.

Wasserman test

Nontreponemal

Antigen (lecitin, cholesterol, and cardiolipin)
Two type serology test for syphilis

nontreponemal screening
treponemal confirmatory

Venereal Disease Research Laboratory (VDRL)
Rapid Plasma Reagin (RPR)
Wasserman reaction modification

T. pallidum haemagglutination assay (TPHA)
T. pallidum passive particle agglutination assay (TPPA)
Microhemagglutination test for T. pallidum (MHA-TP)
Fluorescent treponemal antibody absorption test (FTA-ABS)

There are RPR and TPHA test which available in Dr. Cipto Mangunkusumo Hospital, Jakarta

Rapid Plasma Reagin (RPR)

Treponema Pallidum Haemagglutination Assay (TPHA)

For syphilis diagnosis using serum sample

There is currently no known prevalence of neurosyphilis in dr. Cipto Mangunkusumo Hospital Jakarta and serologic profile of Treponema pallidum from CSF sample
Materials and method

Cross sectional

Clinical pathology and neurology department dr. Cipto Mangunkusumo hospital Jakarta

November 2017 – March 2018

50 CSF sample and serum, patients suspected intracranial infection

Ethical clearance is approved

Data were recorded in Microsoft Excel 2106 (United States), data analysis using International Business Machine (IBM) Statistical Product and Service Solution (SPSS) version 20.0.
Inclusion criteria

• Age more than 18 years
• Clinical information suspected intracranial infection/ brain infection diagnosed by doctor

Exclusion criteria

• Neoplasms: *Acute Myeloid Leukemia*, *Acute Lymphoblastic Leukemia*
• CSF or serum volume is not enough for research
Rapid test *Treponema pallidum*

- Standard Q syphilis Ab test (SD Biosensor, Korea)

Rapid Plasma Reagin (RPR)

- AIM RPR™ Test (PT. Akurat Intan Madya, Jakarta)

*Treponema pallidum* Haemagglutination Assay (TPHA)

- TPHA test kit Plasmatec (Lab21 Healthcare Ltd, UK)
**Inclusion criteria**
- 10 µL CSF rapid test *Treponema pallidum*

**Exclusion criteria**
- 150 µL Serum RPR and TPHA
- 150 µL CSF RPR and TPHA

**Not in research**
- HIV

**CSF analysis**

**Data Analysis**

**Secondary data**

**Research flow**
## Characteristics of the 50 subjects with intracranial infections

| Characteristics                     | Categories | Results (n,%)
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15-19</td>
<td>2 (4)</td>
</tr>
<tr>
<td></td>
<td>20-24</td>
<td>7 (14)</td>
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<tr>
<td></td>
<td>25-29</td>
<td>3 (6)</td>
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<tr>
<td></td>
<td>30-34</td>
<td>11 (22)</td>
</tr>
<tr>
<td></td>
<td>35-39</td>
<td>6 (12)</td>
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<tr>
<td></td>
<td>40-44</td>
<td>3 (6)</td>
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<tr>
<td></td>
<td>45-54</td>
<td>11 (22)</td>
</tr>
<tr>
<td></td>
<td>55-64</td>
<td>5 (10)</td>
</tr>
<tr>
<td></td>
<td>≥ 65</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Gender</td>
<td>Men</td>
<td>34 (68)</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>16 (32)</td>
</tr>
<tr>
<td>Clinical diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tuberculosis meningitis</td>
<td>22 (44)</td>
</tr>
<tr>
<td></td>
<td>Neurosyphilis/suspected</td>
<td>6 (12)</td>
</tr>
<tr>
<td></td>
<td>Intracranial infection</td>
<td>6 (12)</td>
</tr>
<tr>
<td></td>
<td>Myelitis</td>
<td>3 (6)</td>
</tr>
<tr>
<td></td>
<td>Meningitis</td>
<td>2 (4)</td>
</tr>
<tr>
<td></td>
<td>Viral encephalitis</td>
<td>2 (4)</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>9 (18)</td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loss of consciousness</td>
<td>13 (26)</td>
</tr>
<tr>
<td></td>
<td>Behavioral changes</td>
<td>7 (14)</td>
</tr>
<tr>
<td></td>
<td>Headache</td>
<td>7 (14)</td>
</tr>
<tr>
<td></td>
<td>Blurry vision/hearing loss</td>
<td>6 (12)</td>
</tr>
<tr>
<td></td>
<td>Seizure</td>
<td>4 (8)</td>
</tr>
<tr>
<td></td>
<td>Paresis</td>
<td>4 (8)</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>9 (18)</td>
</tr>
</tbody>
</table>
Subject characteristics based on age and gender

Total 104 samples

Inclusion criteria

50 samples

Characteristics of 50 subjects

Men

Women

AGE (years)

≥65
55-64
45-54
40-44
35-39
30-34
25-29
20-24
15-19

Men

34 (68%)

Women

16 (32%)

39.02 ± 12.76 years
## Clinical diagnosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis meningitis</td>
<td>22 (44)</td>
</tr>
<tr>
<td>Neurosyphilis/suspected Infection</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Intracranial infection</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Myelitis</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Viral encephalitis</td>
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</tr>
<tr>
<td>Others</td>
<td>9 (18)</td>
</tr>
</tbody>
</table>

- **Cryptococcal meningitis**
- CMV
- Neuromyelitis optica
- Tetraparesis
- Medulloblastoma
- Guillain Barre Syndrome
- Multiple Sclerosis
- Motor neuron disease
- Idiopathic

Similar with Ganiem et al\(^{32}\), Tb meningitis prevalence in Bandung, there were 83% adults patients with TB meningitis.

WHO→ Indonesia, second country after India, which has high incidence of TB case in 2016.
### Clinical Symptoms

- **Loss of consciousness**: 13 (26)
- **Behaviorsal changes**: 7 (14)
- **Headache**: 7 (14)
- **Blurry vision/hearing loss**: 6 (12)
- **Seizure**: 4 (8)
- **Paresis**: 4 (8)
- **Others**: 9 (18)

**Fever**
- Urination disturbances
- No data

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### Cerebrospinal fluid

- **Kriteria CDC**
  - 4 confirmed neurosyphilis
  - 3 presumptive neurosyphilis

### Serum

- 14% from 50 subjects
Mean age of neurosyphilis: 39 ± 13.81 years

Wöhrl et al. mean age of neurosyphilis patients: 46.7 ± 14.8 years

CDC Sexually Transmitted Disease Surveillance 2016:
- 88.9% men with primary and secondary syphilis

Wöhrl et al., proportion 38 men (88.4%) and 5 women (11.6%)

Bergemann et al. in South Africa:
- Proportion 18 men (81.8%) and 4 women (18.2%)

Gender distribution:
- 85.71% Men
- 14.29% Women
Neurosyphilis subjects based on age and gender

AGE (years)

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Men (%)</th>
<th>Women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-19</td>
<td>14.29%</td>
<td></td>
</tr>
<tr>
<td>20-24</td>
<td></td>
<td>28.56%</td>
</tr>
<tr>
<td>25-29</td>
<td></td>
<td>14.29%</td>
</tr>
<tr>
<td>30-34</td>
<td>28.56%</td>
<td></td>
</tr>
<tr>
<td>35-39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55-64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CDC 2016: Age 30-34 years increased 20.1%, age 55-64 years 25%, age 20-24 years about 8.1%, age 25-29 years about 19.6%, and age 40-44 years about 12.8%.32
Clinical symptoms of neurosifilis

- **4 Blurry vision**: 57.13%
- **1 Behavioral changes**: 14.29%
- **1 Headache**: 14.29%
- **1 No data**: 14.29%

Marra et al\(^3\)\(^7\) two groups with positive CSF VDRL:
- **Group 1**: 27 patients (46.6%) with eyes disturbances/hearing loss
- **Group 2**: 19 patients (40.4%) with eyes disturbances/hearing loss

Vanhaecke et al\(^3\)\(^8\) 40 patients:
- 26 (65%) patients with eyes disturbances,
- 24 (60%) with neurological symptoms,
- 10 (25%) both;
- 16 (40%) patients only eyes disturbances and 14 (35%) only neurological symptoms.
## Cerebrospinal fluid analysis profile between neurosyphilis and other intracranial infections

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Neurosyphilis (n=7)</th>
<th>Other intracranial infections (n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Colorless</td>
<td>Colorless</td>
</tr>
<tr>
<td>Clarity</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>Clot</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Cell count (cell/µL)</td>
<td>12.71 ± 9.20</td>
<td>10 (1-495)</td>
</tr>
<tr>
<td>PMN (cell/µL)</td>
<td>1.14 ± 0.69</td>
<td>3 (0-174)</td>
</tr>
<tr>
<td>MN (cell/µL)</td>
<td>11.57 ± 9.47</td>
<td>7 (0-456)</td>
</tr>
<tr>
<td>Cryptococcal (India ink)</td>
<td>Not found</td>
<td>Found/Not found</td>
</tr>
<tr>
<td>Nonne</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Pandy</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>CSF protein (mg/dL)</td>
<td>49.29 ± 21.49</td>
<td>45 (10-650)</td>
</tr>
<tr>
<td>CFS glucose (mg/dL)</td>
<td>55 ± 5.16</td>
<td>61.72 ± 21.19</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>101.04 ± 20.10</td>
<td>102.90 (64-223)</td>
</tr>
<tr>
<td>CSF chloride (mEq/L)</td>
<td>122.14 ± 2.48</td>
<td>116.33 (98-130)</td>
</tr>
</tbody>
</table>
Found *Cryptococcus* in cytospin slide CSF analysis CSF sample no 42 (Wright stain, 40x10), black arrow
## Proportion CSF analysis rapid test *Treponema pallidum*, RPR, and TPHA in intracranial infections

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Categories</th>
<th>Results (n%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell count (cell/µL)</td>
<td>&lt; 5</td>
<td>16 (32)</td>
</tr>
<tr>
<td></td>
<td>5≤cell≤20</td>
<td>14 (28)</td>
</tr>
<tr>
<td></td>
<td>&gt;20</td>
<td>20 (40)</td>
</tr>
<tr>
<td>Cell domination</td>
<td>Polymorphonuclear</td>
<td>11 (22)</td>
</tr>
<tr>
<td></td>
<td>Mononuclear</td>
<td>33 (66)</td>
</tr>
<tr>
<td></td>
<td>Proportional PMN and MN</td>
<td>6 (12)</td>
</tr>
<tr>
<td>CSF protein (mg/dL)</td>
<td>≤ 45</td>
<td>26 (52)</td>
</tr>
<tr>
<td></td>
<td>&gt; 45</td>
<td>24 (48)</td>
</tr>
<tr>
<td>Cryptococcal (India ink)</td>
<td>Found</td>
<td>2 (4)</td>
</tr>
<tr>
<td></td>
<td>Not found</td>
<td>48 (96)</td>
</tr>
</tbody>
</table>
### Parameter Categories and Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Categories</th>
<th>Results (n%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid test <em>Treponema pallidum</em></td>
<td>Reactive</td>
<td>4 (8)</td>
</tr>
<tr>
<td></td>
<td>Nonreactive</td>
<td>46 (92)</td>
</tr>
</tbody>
</table>

- **Reactive**: a red line at control line and at test line

- **Reactive rapid test *Treponema pallidum***
  - no sample 24 (a)
  - no sample 45 (b)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Categories</th>
<th>Results (n%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF RPR</td>
<td>Reactive</td>
<td>4 (8)</td>
</tr>
<tr>
<td></td>
<td>Nonreactive</td>
<td>46 (92)</td>
</tr>
<tr>
<td>Serum RPR</td>
<td>Reactive</td>
<td>8 (16)</td>
</tr>
<tr>
<td></td>
<td>Nonreactive</td>
<td>42 (84)</td>
</tr>
</tbody>
</table>

Reactive = flocculation carbon particles in antigen suspension

CSF RPR test
Circle no 1 positive control
Circle no 2 negative control

CSF sample no 24 (a)
Circle no 1 CSF RPR titre 1:2 (b)
Serum RPR test
Circle no 1 positive control
Circle no 2 negative control
Circle no 3 serum sample no 24 (a)
Circle no 8 serum RPR titre 1: >10240 (b)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Categories</th>
<th>Results (n%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF TPHA</td>
<td>Reactive</td>
<td>4 (8)</td>
</tr>
<tr>
<td></td>
<td>Nonreactive</td>
<td>46 (92)</td>
</tr>
<tr>
<td>Serum TPHA</td>
<td>Reactive</td>
<td>9 (18)</td>
</tr>
<tr>
<td></td>
<td>Nonreactive</td>
<td>41 (82)</td>
</tr>
</tbody>
</table>

Reactive = agglutination in test cells and no agglutination in control cells

Red box (positive control)
Blue box (negative control)
Green box (reactive CSF TPHA no sample 24)
Orange box (reactive serum TPHA no sample 45)
Yellow box (reactive CSF TPHA no sample 45)
**Confirmed neurosyphilis**

- Syphilis any stages
- Reactive CSF VDRL

**Presumptive neurosyphilis**

- Syphilis any stages
- Nonreactive CSF VDRL
- CSF pleositosis/protein increased
- Clinical symptoms consistent with syphilis without alternative diagnosis

2 HIV positive
1 unknown

<table>
<thead>
<tr>
<th>Neurosyphilis</th>
<th>3 Presumptive neurosyphilis</th>
<th>4 Confirmed neurosyphilis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 Presumptive neurosyphilis</td>
<td>4 Confirmed neurosyphilis</td>
</tr>
<tr>
<td></td>
<td>42.86%</td>
<td>57.14%</td>
</tr>
</tbody>
</table>

85.71% of neurosyphilis in this research HIV positive

All HIV positive

Confirmed neurosyphilis
Presumptive neurosyphilis
Conclusions

Neurosyphilis was found 14% of our patient population.

85.71% was reactive for HIV.
THANK YOU