Has CXCL13 an added value in the diagnosis of neurosyphilis?

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Running title: Added value of CXCL13 as a Biomarker for Neurosyphilis

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ABSTRACT
In patients with syphilis, central nervous system (CNS) involvement is often difficult to determine. In patients who also are infected with human immunodeficiency virus (HIV), this is even more challenging as cerebrospinal fluid (CSF) pleocytosis can be attributed to HIV, syphilis or both. Hence, this study investigated 1) CSF-CXCL13 as a potential marker to diagnose neurosyphilis in HIV-infected individuals, 2) the added value of CSF-CXCL13 to conventional CSF biomarkers such as Rapid Plasma Reagin (RPR) in diagnosing neurosyphilis. We included 103 syphilis patients from two centers in The Netherlands, 47 non-HIV patients and 56 HIV-infected patients. A positive CSF-RPR was regarded as the gold standard for neurosyphilis. CSF-CXCL13 levels were significantly higher in neurosyphilis patients when neurosyphilis is diagnosed by CSF-RPR (p=0.0002) compared to their syphilis control group. The sensitivity and specificity of CSF-CXCL13 (cut-off 76.3 pg/ml) to diagnose neurosyphilis using positive CSF-RPR as gold standard were 50% and 90%, respectively. CSF-CXCL13 had an added value to CSF-RPR positivity in 70% of HIV-positive patients and in 33% of HIV-negative patients. Our data show that CSF-CXCL13 might be a potential additional marker in neurosyphilis when other markers are not conclusive. The added value of CSF-CXCL13 measurement to the current neurosyphilis gold standard appears to benefit HIV-positive patients more than HIV-negative patients.
**Introduction**

In 2007 the World Health Organization estimated an incidence of 12 million new infections with *Treponema pallidum* (*T. pallidum*) each year worldwide (11). Invasion of the central nervous system (CNS) by *T. pallidum* may occur during any disease stage of syphilis, leading to development of neurosyphilis in some of the patients (6, 7). When dual infections with HIV and syphilis exist, the diagnostic challenge increases. A positive cerebrospinal fluid (CSF) Venereal Disease Research Laboratory (VDRL) is generally considered as the gold standard for neurosyphilis (1), however several studies have clearly shown that a positive CSF- Rapid Plasma Reagin (RPR) is an alternative for CSF-VDRL (3, 16) and RPR is also recommended by European guidelines of 2014 (IUSTI-2014) (5). When the CSF-RPR or CSF-VDRL is negative, the diagnosis relies on other markers like CSF-pleocytosis, CSF *T. pallidum* particle agglutination (TPPA) index and clinical signs and symptoms.

As the laboratory diagnosis of neurosyphilis is difficult, new markers are needed and CSF B cell chemoattractant, chemokine (C-X-C motif) ligand 13 (CXCL13) is currently forwarded as an interesting marker. CXCL13 has been demonstrated to be elevated in B lymphocyte rich CSF (12, 14). CSF-CXCL13 has a higher sensitivity when compared to the established diagnostic markers for neuroborreliosis, such as CSF-pleocytosis and *Borrelia* specific antibodies (13). High numbers of B lymphocytes have also been observed in CSF from patients with syphilitic meningitis (8). CXCL13 dictates homing and motility of B cells in
lymphoid tissue (10), and it plays a key role in the migration of B cells into the CSF (14). Hence, studies of CSF-CXCL13 as a diagnostic marker for neurosyphilis are warranted. Recently it has been shown that CSF-CXCL13 concentration was particularly useful for the diagnosis of neurosyphilis in HIV-infected patients independent of CSF-pleocytosis and markers of HIV disease (9). So far, there is only one study available.

Therefore, we aimed to 1) investigate CSF-CXCL13 as a potential marker for neurosyphilis in HIV-infected and HIV-seronegative individuals, and 2) to investigate the added value of CSF-CXCL13 to CSF-RPR in diagnosing neurosyphilis.

**Materials and methods**

**Study population**

One hundred and seven patients from two centers in The Netherlands (VU University Medical Center (VUMC) and Radboud University Medical Center), were selected in the period of March 2005 till February 2012. The study included patients with confirmed syphilis on the base of a positive TPPA test. One hundred three patients underwent an of HIV test, 56 of them were HIV-seropositive and 47 were HIV-seronegative. The remaining 4 patients without HIV test were excluded from this study. All of the 103 patients were subjected to a lumbar puncture, therefore, routine CSF samples were used for the quantification of CXCL13. The CSF samples were kept frozen at −80°C, and all sample and patients history were anonymized making it impossible to trace back
the patients for further clinical data. Patient characteristics are depicted in Table 1. In this study we considered a positive CSF-RPR as the gold standard for diagnosing neurosyphilis (3, 16). According to the Dutch legislature (Evaluation of the Dutch Medical treatment Act (WGBO)) our study qualified for ethical clearance exemption since it is a non-profit, scientific study and its purpose is to improve the quality of diagnosis.

Laboratory tests

CXCL13 was measured in CSF using the Human CXCL13/BLC/BCA-1 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN) according to manufacturer's instructions. The intra-assay coefficient of variation (CV) was between 1.4% and 4.3%; the inter-assay CV was higher (26%), but could only be measured at the low end of the calibration curve (due to insufficient CSF samples with high CXCL13 concentration). Recovery was between 95% and 105%. The assay was linear up to 1000 pg/ml. Lower detection limit was 7.8 pg/ml. Samples below detection limit were recoded as zero (0) pg/ml.

White blood cell count was performed using standard flow cytometrical immunophenotyping.

RPR in CSF was measured using the RPR card test (Biokit, S.A, Barcelona, Spain). TPPA test (Fujirebio Inc, Japan) was used to test specific treponemal antibody in CSF.

Immunonephelometry using a Beckman Immage nephelometer was used to measure CSF and serum albumin.
Statistical Analysis

Data analysis was performed using SPSS (SPSS 20, Inc. Chicago, Illinois, USA). CXCL13 levels were compared between different patient groups (patients with and without HIV, and patients with and without neurosyphilis), using the Mann-Whitney U test statistics. The sensitivity and specificity of CSF-CXCL13 was calculated using a 2X2 table. The added value was taken as a percentage of non-neurosyphilis patients (negative by CSF-RPR) that tested positive for CSF-CXCL13 with a cut-off value ≥ 76.3 pg/ml divided by the total number of patients with a positive CSF-RPR. The cut-off for clinical significant level of CXCL13 was defined as any value above the 25th percentile of the positive CXCL13 values and thereby set at 76.3 pg/ml. A p-value <0.05 was considered statistically significant.

Results

Patient characteristics

Baseline characteristics of the study population are summarized in Table 1. The study included 103 syphilis patients with median age of 44 years, 56 were HIV-infected and 47 HIV-seronegative. The CSF-RPR was positive in 6/47 (13%) of HIV-negative and in 10/56 (18%) of HIV-positive patients. Sixteen percent of all patients were categorized as neurosyphilis according to a positive CSF-RPR. Clinical signs or symptoms were present in 33 (39%) patients, these included neurological, psychiatric, acoustic and ophthalmological signs and symptoms.
Fifty-two patients (61%) had no neurological or psychiatric symptoms, while for 19 patients clinical data were missing.

In all patients, CSF-CXCL13 levels were significantly higher in patients with established neurosyphilis as diagnosed by positive CSF-RPR (median 177 pg/ml, p=0.0002) compared to patients with syphilis but without CNS involvement (median 0 pg/ml) (Fig. 1a). CSF-CXCL13 levels were similar in all HIV-infected and non-HIV-infected patients (both medians were 0 pg/ml, p=0.16). However, CSF-CXCL13 levels were elevated in HIV (median 177 pg/ml, p=0.014) and non-HIV (median 783 pg/ml, p=0.005) patients with neurosyphilis when compared to their syphilis control groups (both medians were 0 pg/ml) (Fig. 1b).

Regarding RPR-positivity as gold standard, a sensitivity of 50% and a specificity of 90% were obtained when using the cut-off of 76.3 pg/ml CSF-CXCL13 as marker for the diagnosis of neurosyphilis.

The assumed added value of CSF-CXCL13 to CSF-RPR positivity was 9/16 (56%). The added value is higher in HIV-positive patients 7/10 (70%) as compared to HIV-negative patients 2/6 (33%).
Discussion

The diagnosis of asymptomatic neurosyphilis is based on CSF abnormalities, but in individuals infected by both syphilis and HIV the diagnosis may be difficult because of overlapping diagnostic test results. In this study, we demonstrated significantly elevated levels of CSF-CXCL13 in neurosyphilis patients. These data support the observations made by Marra at el (9). Using a cut-off level of 76.3 pg/ml (positive CXCL13 levels > 25th percentile), we estimated the added value of CSF-CXCL13 as a diagnostic marker for neurosyphilis to be higher in HIV patients than HIV-negative patients for diagnosing neurosyphilis.

Only one study similar to ours has been performed earlier, in this study they included HIV patients only (9). Unlike Marra et al. (9), we included both HIV-infected and non-HIV-infected patients. We clearly showed the added value of CSF-CXCL13 to CSF-RPR and we also showed that HIV-infected patients benefited more from CSF-CXCL13 as an added marker for diagnosis of neurosyphilis. Cepok et al., showed that HIV-infection triggers an early profound B cell response in CNS, which serves as the main virus-related B cell subset in the CSF (4), and B cells are the main source of CSF-CXCL13 (9, 12). However, Bremell et al. showed that CSF-CXCL13 was not increased in HIV-infected patients (2) and we did not observe elevated CSF-CXCL13 in dual HIV and T. pallidum infected control group with a negative CSF-RPR. Even though the effect of HIV alone was not proven in our study, Bremell et al. (2) data may suggests
that there is an added CSF-CXCL13 release in neurosyphilis patients co-infected with HIV. Hence, the observed added benefit of CSF-CXCL13 in HIV-infected patients in diagnosing neurosyphilis. However, we cannot strictly exclude other causes for high or detectable CXCL13.

Marra and colleagues analyzed two CXCL13 cut-offs; the lower cut-off (10 pg/ml) showed a high sensitivity and the higher cut-off (250 pg/ml) showed a high specificity (9). In our study, 76.3 pg/ml is a reasonable cut-off value and can be used to distinguish between patients with and without neurosyphilis. The 76.3 pg/ml cut-off showed a sensitivity and specificity of 50% and 90% respectively. Even though CSF-CXCL13 is not a confirmatory test, we assume on the basis of serological syphilis and pathogenesis of CXCL13 that CSF-CXCL13 can contribute to the diagnosis of neurosyphilis. Furthermore, the use of CSF-CXCL13 for diagnosis of neurological infections with spirochetes, such as neuroborreliosis and also neurosyphilis, has been shown before (9, 15).

However, the current study demonstrates an added value of CXCL13 as a marker in the diagnosis of neurosyphilis, which increases in HIV-infected patients.

A limitation of this study is that we only could include patients in whom CSF was obtained. Syphilis patients and HIV patients without suspected neurological disease were not included, implying possible selection bias. However, our data did not find any difference in CXCL13 levels between patients with and without neurological symptoms, implying that this bias probably is not that important. Lack of clinical data, such as treatment for syphilis or treatment
with drugs active against *T. pallidum* or other etiologies that may affect CSF-
CXCL13 levels, also limits our study.

In conclusion, CSF-CXCL13 may be a potential marker for neurosyphilis
as demonstrated by elevated levels in patients with suspected neurosyphilis
according to a positive CSF-RPR. The added value of CSF-CXCL13 in the
diagnosis of neurosyphilis above the easy and cheap to perform CSF-RPR test,
benefits HIV-positive patients more than non-HIV patients.
Conflict of Interest

No competing financial interests exist.

Acknowledgements

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References


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FIG 1 (a) CXCL13 levels (pg/ml) in patients diagnosed as neurosyphilis by a positive CSF-RPR. (b) CSF-CXCL13 levels in HIV positive and HIV negative patients diagnosed with syphilis and neurosyphilis. Horizontal dotted line shows cut-off of 76.3 pg/ml and the short solid horizontal lines show CSF-CXCL13 median levels.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Syphilis and neurosyphilis patients (n=103)</th>
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<tbody>
<tr>
<td>Sex</td>
<td>93 males (90%)</td>
</tr>
<tr>
<td>Age (median, min-max)</td>
<td>44 yrs. (20-84)</td>
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<tr>
<td>HIV status</td>
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<tr>
<td>-Positive</td>
<td>56 (54%)</td>
</tr>
<tr>
<td>-Negative</td>
<td>47 (46%)</td>
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<tr>
<td>HIV load (median, min-max)</td>
<td>22<em>10E3 copies/ml (20-300</em>10E4)</td>
</tr>
<tr>
<td>CSF-CXCL13 (median, min-max)</td>
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</tr>
<tr>
<td>-HIV-positive</td>
<td>0 pg/ml (0-15480)</td>
</tr>
<tr>
<td>-HIV-negative</td>
<td>0 pg/ml (0-11598)</td>
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<td>CSF pleocytosis (median, min-max)</td>
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<tr>
<td>-HIV-positive</td>
<td>5 cells/mm3 (0-150)</td>
</tr>
<tr>
<td>-HIV-negative</td>
<td>1 cells/mm3 (0-222)</td>
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<tr>
<td>CSF Protein (median, min-max)</td>
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<tr>
<td>-HIV-positive</td>
<td>406 mg/L (150-1511)</td>
</tr>
<tr>
<td>-HIV-negative</td>
<td>398 mg/L (150-3088)</td>
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<td>Reactive serum-RPR test (≥1:4)</td>
<td>74/103 (72%)</td>
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<tr>
<td>Reactive CSF-RPR test (≥1:2)</td>
<td>16/103 (16%)</td>
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