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Has CXCL13 an Added Value in Diagnosis of Neurosyphilis?

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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 (i) CSF chemokine (C-X-C motif) ligand 13 (CXCL13) as a potential marker to diagnose neurosyphilis in HIV-infected individuals and (ii) the added value of CSF CXCL13 to conventional CSF biomarkers, such as the rapid plasma reagin test (RPR), in diagnosing neurosyphilis. We included 103 syphilis patients from two centers in The Netherlands: 47 non-HIV-infected 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 was diagnosed by CSF-RPR ($P = 0.0002$) than in the syphilis control group. The sensitivity and specificity of CSF CXCL13 (cutoff of 76.3 pg/ml) to diagnose neurosyphilis by using positive CSF-RPR as the 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.

In 2007, the World Health Organization estimated an incidence of 12 million new infections with *Treponema pallidum* each year worldwide (1). Invasion of the central nervous system (CNS) by *T. pallidum* may occur during any disease stage of syphilis, leading to the development of neurosyphilis in some patients (2, 3). When dual infections with HIV and syphilis exist, the diagnostic challenge increases. A positive cerebrospinal fluid (CSF) Venereal Disease Research Laboratory test (VDRL) is generally considered the gold standard for neurosyphilis (4); however, several studies have clearly shown that a positive CSF rapid plasma reagin test (RPR) is an alternative to CSF-VDRL (5, 6), and RPR is also recommended by 2014 European guidelines (IUSTI-2014) (7). When the CSF-RPR or CSF-VDRL is negative, the diagnosis relies on other markers, like CSF pleocytosis, the 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 (8, 9). CSF CXCL13 has a higher sensitivity than the established diagnostic markers for neuroborreliosis, such as CSF pleocytosis and *Borrelia*-specific antibodies (10). High numbers of B lymphocytes have also been observed in CSF from patients with syphilitic meningitis (11). CXCL13 dictates homing and motility of B cells in lymphoid tissue (12), and it plays a key role in the migration of B cells into the CSF (9). Hence, studies of CSF CXCL13 as a diagnostic marker for neurosyphilis are warranted. Recently, it has been shown that the CSF CXCL13 concentration was particularly useful for the diagnosis of neurosyphilis in HIV-infected patients independent of CSF pleocytosis and markers of HIV disease (13). So far, there is only one study available.

Therefore, we aimed to (i) investigate CSF CXCL13 as a potential marker for neurosyphilis in HIV-infected and HIV-seronegative individuals and (ii) 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 to February 2012. The study included patients with confirmed syphilis on the basis of a positive TPPA test. One hundred and three patients underwent an HIV test, and 56 of them were HIV seropositive and 47 were HIV seronegative. The remaining 4 patients without an 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 samples and patients histories 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 (5, 6). According to the Dutch legislature (Evaluation of the Dutch Medical Treatment Act

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TABLE 1 Patients' characteristics

Characteristic of syphilis and neurosyphilis patients (<i>n</i> = 103)	Value
No. (%) of males	93 (90)
Median (minimum–maximum) age in yrs	44 (20–84)
No. (%) of patients by HIV status	
Positive	56 (54)
Negative	47 (46)
Median (minimum–maximum) HIV load in copies/ml	22 × 10E3 (20–300 × 10E4)
Median (minimum–maximum) CSF CXCL13 concn in pg/ml	
HIV-positive patients	0 (0–15,480)
HIV-negative patients	0 (0–11,598)
Median (minimum–maximum) CSF pleocytosis in cells/mm ³	
HIV-positive patients	5 (0–150)
HIV-negative patients	1 (0–222)
Median (minimum–maximum) CSF protein concn in mg/liter	
HIV-positive patients	406 (150–1,511)
HIV-negative patients	398 (150–3,088)
No. (%) of patients positive by reactive serum-RPR (≥1:4)	74 (72)
No. (%) of patients positive by reactive CSF-RPR (≥1:2)	16 (16)

[WGBO]), our study qualified for ethical clearance exemption, since it is a nonprofit, 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 enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. The intraassay coefficient of variation (CV) was between 1.4% and 4.3%; the interassay CV was higher (26%) but could be measured only at the low end of the calibration curve (due to insufficient CSF samples with high CXCL13 concentrations). Recovery was between 95% and 105%. The assay was linear up to 1,000 pg/ml. The lower detection limit was 7.8 pg/ml. Samples below the detection limit were recoded as 0 pg/ml.

The white blood cell count was performed by using standard flow cytometric immunophenotyping.

Rapid plasma reagin in CSF was measured using the rapid plasma reagin card test (Biokit S.A., Barcelona, Spain). A 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, IL, USA). CXCL13 levels were compared between different patient groups (patients with and without HIV and patients with and without neurosyphilis), using Mann-Whitney U test statistics. The sensitivity and specificity of CSF CXCL13 were calculated using a 2-by-2 table. The added value was taken as a percentage of nonneurosyphilis patients (negative by CSF-RPR) that tested positive for CSF CXCL13 with a cutoff value of ≥76.3 pg/ml divided by the total number of patients with a positive CSF-RPR. The cutoff for the clinical significance 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 of <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 a median age of 44 years, 56 were HIV infected, and 47 were HIV seronegative. The CSF-RPR was positive in 6/47 (13%) HIV-negative patients and in 10/56 (18%) HIV-positive patients. Sixteen percent of all patients were categorized as neurosyphilis positive 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 of 177 pg/ml, *P* = 0.0002) than in patients with syphilis but without CNS involvement (median of 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-infected (median of 177 pg/ml, *P* = 0.014) and non-HIV-infected (median of 783 pg/ml, *P* = 0.005) patients with neurosyphilis compared to those in the syphilis control groups (both medians were 0 pg/ml) (Fig. 1b).

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

The assumed added value of CSF CXCL13 to CSF-RPR positivity was the diagnosis of 9/16 (56%) patients. The added value is higher in HIV-positive patients (7/10 [70%]) than in 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 et al. (13). Using a cutoff level of 76.3 pg/ml (positive CXCL13 levels above the 25th percentile), we estimated the added value of CSF CXCL13 as a diagnostic marker for neurosyphilis to be higher in HIV-positive patients than in HIV-negative patients for diagnosing neurosyphilis.

Only one study similar to ours has been performed, in which the authors included HIV patients only (13). Unlike Marra et al. (13), 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 (14), and B cells are the main source of CSF CXCL13 (8, 13). However, Bremell et al. showed that CSF CXCL13 was not increased in HIV-infected patients (15), and we did not observe elevated CSF CXCL13 concentrations in the HIV- and *T. pallidum*-coinfecting control group with a negative CSF-RPR result. Even though the effect of HIV alone was not proven in our study, Bremell et al. (15) data may suggest that there is an added CSF CXCL13 release in neurosyphilis patients coinfecting with HIV,

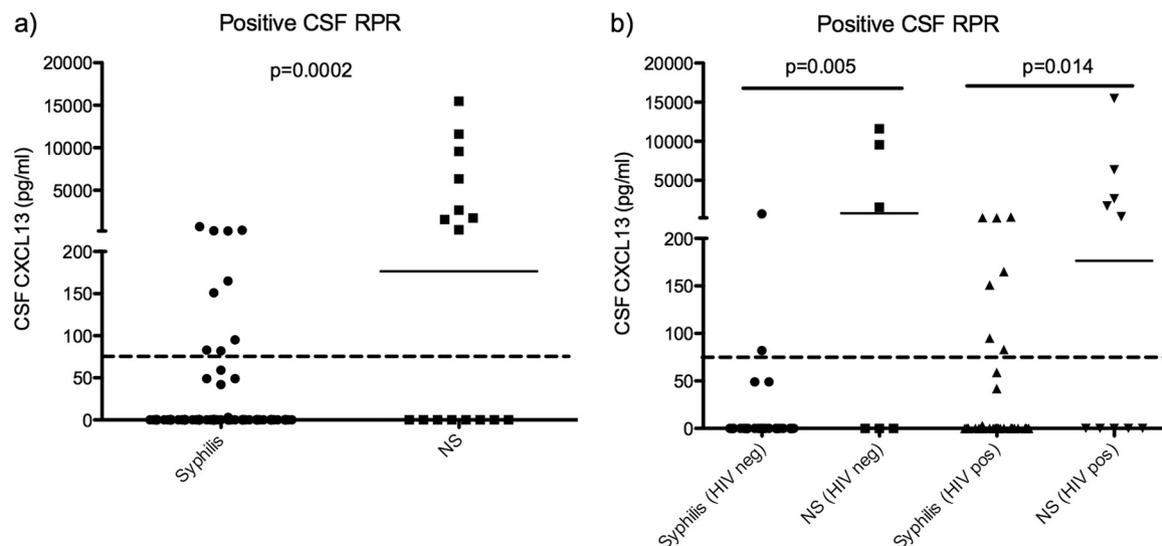


FIG 1 (a) CXCL13 levels (picogram/milliliter) in patients diagnosed as neurosyphilis positive by a positive CSF-RPR; (b) CSF CXCL13 levels in HIV-positive and HIV-negative patients diagnosed with syphilis and neurosyphilis. The horizontal dotted line shows a cutoff of 76.3 pg/ml, and the short, solid horizontal lines show CSF CXCL13 median levels.

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 cutoffs; the lower cutoff (10 pg/ml) showed a high sensitivity, and the higher cutoff (250 pg/ml) showed a high specificity (13). In our study, 76.3 pg/ml is a reasonable cutoff value and can be used to distinguish between patients with and without neurosyphilis. The 76.3 pg/ml cutoff showed a sensitivity and specificity of 50% and 90%, respectively. Even though CSF CXCL13 concentration is not a confirmatory test, we assume on the basis of serological syphilis and pathogenesis of CXCL13 that CSF CXCL13 concentration can contribute to the diagnosis of neurosyphilis. Furthermore, the use of CSF CXCL13 concentration for the diagnosis of neurological infections with spirochetes, such as neuroborreliosis and also neurosyphilis, has been shown before (13, 16). However, the current study demonstrates an added value of CXCL13 concentration as a marker in the diagnosis of neurosyphilis, which increases in HIV-infected patients.

A limitation of this study is that we could include only patients from 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. A 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, in addition to the easy and cheap CSF-RPR, benefits HIV-positive patients more than non-HIV patients.

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