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# CXCL13 levels are more increased in cerebrospinal fluid and plasma of patients with acute infectious than in non-infectious diseases of the central nervous system

## Concentrațiile CXCL13 în lichidul cefalorahidian și plasma pacienților cu infecții acute sunt mai crescute decât în patologii non-infecțioase ale sistemului nervos central

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## Abstract

***Background:*** *During the acute inflammatory process, the CXCL13 chemokine plays an important role in B cell recruitment within the central nervous system (CNS).*

***Objective:*** *The objective of the study consisted of the evaluation of CXCL13 chemokine cerebral spinal fluid (CSF) and plasma levels in patients with acute infectious and non-infectious neurological diseases correlated with pleocytosis and CSF protein levels.*

***Material and method:*** *This retrospective study was conducted over one year and included 72 patients. Thirty-eight patients (52.8%) suffering from infectious neurological disease, acute viral and bacterial meningitis, meningoencephalitis, and 34 patients (44.2%) diagnosed with non-infectious neurological diseases.*

*CXCL13 chemokine CSF and plasma levels were determined through the ELISA technique with the Human CXCL13/BLC/BCA-1 kit. CSF cell count, glucose and protein levels, along with anti-Borrelia burgdorferi antibodies were monitored using the ELISA technique.*

***Results:*** *CXCL13 chemokine levels in the CSF of patients with acute infectious neurological diseases showed a median value of 23.07 pg/mL, which was significantly higher in comparison with the median value of 11.5 pg/mL of patients with noninfectious neurological diseases (p-0.03). CXCL13 median plasma concentration in patients with infectious neurological diseases was 108.1 pg/mL, in comparison with the second patient category, 50.7 pg/ml (p-0.001). We observed a statistically significant association between CXCL13 concentrations, CSF cell count and proteins. The higher the CXCL13 chemokine level, the more increased the cell count was.*

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***Conclusions:*** *CXCL13 levels in the CSF was significantly increased in patients with acute infectious neurological diseases compared with patients with non-infectious diseases. Moreover, CXCL13 chemokine concentration was significantly correlated with the number of cells and proteins in the CSF of patients suffering from neuroinfections.*

***Keywords:*** *CXCL13, cerebrospinal fluid, diagnosis, neuroinflammation.*

## Rezumat

*Chemokina CXCL13 joacă un rol important în recrutarea celulelor B la nivelul sistemului nervos central (SNC) în perioada procesului inflamator acut.*

***Obiectivul*** *studiului a constat în evaluarea nivelului chemokinei CXCL13 din lichidul cefalorahidian (LCR) și plasmă, la pacienții cu afecțiuni neurolgice acute infecțioase și non – infecțioase, în corelație cu pleiocitoza și concentrația proteinelor din LCR.*

***Material și metodă:*** *studiul retrospectiv s-a efectuat pe o perioadă de un an și a inclus un număr de 72 pacienți. 38 pacienți (52.8%) cu boli neurologice infecțioase, meningite, meningoencefalite acute virale, bacteriene și 34 pacienți (47.2%) cu boli neurologice non-infecțioase. Nivelul chemokinei CXCL13 din LCR și plasmă a fost determinat prin technica ELISA cu ajutorul kitului Human CXCL13/ BLC/BCA-1. S-au monitorizat pleiocitoza LCR, glicorahia, proteinorahia. Anticorpii anti Borrelia burgdorferi, s-au efectuat utilizând tehnica ELISA.*

***Rezultate:*** *nivelul chemokinei CXCL13 din LCR la pacienții cu afecțiuni neurologice acute infecțioase a prezentat valori mediane de 23.07 pg/ml, mai ridicate comparativ cu mediana de 11.5 pg/mL la pacienții cu afecțiuni neurologice non-infecțioase (p-0.03). Valoarea mediană a concentrației plasmatice a CXCL13 la pacienții cu afecțiuni neurologice infecțioase a fost de 108.1 pg/mL comparativ cu a doua categorie de pacienți, 50.7 pg/mL (p-0.001). S-a remarcat o asociere semnificativ statistică între concentrațiile CXCL13, pleiocitoza și proteinele din LCR. Nivelul chemokinei CXCL13 a fost cu atât mai ridicat cu cât pleiocitoza a fost mai crescută.*

***Concluzii:*** *Nivelele de CXCL13 din LCR au avut o valoare semnificativ crescută la pacienții cu boli neurologice acute infecțioase comparativ cu bolile non-infecțioase. Concentrația CXCL13 din LCR s-a corelat semnificativ cu numărul de elemente celulare și proteinorahia la pacienții cu neuroinfecții.*

***Cuvinte cheie:*** *CXCL13, lichid cefalorahidian, diagnostic, neuroinflamație.*

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## Introduction

Chemokines and cytokines play an important role in the recruitment of B cells to the central nervous system (CNS) compartment during neuroinflammation.

CXCL13 chemokine (BCA-13 a B cell-attracting chemokine-1) belongs to the CXC chemokine family. It selectively attracts B lymphocytes and T helper cells via the CXCR5 chemokine receptor. CXCL13 is a chemokine involved in the development and maintenance of secondary lymphoid organs. During inflammatory processes, CXCL13 is also expressed in non-lymphoid tissues, such as the CNS, where it functions as an attractor of B cells [1].

It was also noticed that in the case of chronic inflammatory brain disorders CXCL13 plays an important role in the formation of ectopic lymphoid tissues within the CNS.

Patients with multiple sclerosis (MS), Lyme neuroborreliosis (LNB), and other autoimmune diseases present high levels of CXCL13 expression in the cerebrospinal fluid (CSF), which correlate with the number of B and T cells in the CSF and intrathecal immunoglobulin production [2-4].

Recent studies have demonstrated that, in exceptional cases, patients suffering from acute aseptic meningitis who also develop meningoencephalitis have significantly higher levels of CXCL13 compared with patients with aseptic meningitis (5). Thus, it is considered that CXCL13 concentration in CSF can be a useful

prognostic marker for aseptic meningitis. High levels of CXCL13 in CSF have been detected in cases of Human African trypanosomiasis (HAT), also known as sleeping sickness, primary CNS lymphoma, infection with human immunodeficiency virus (HIV), cryptococcal meningitis, tuberculosis, neurosyphilis, and autoimmune diseases [4, 5].

Chemokine CXCL13 has recently been suggested as a prognostic marker for multiple sclerosis (MS) and clinically isolated syndrome (CIS). It has also been observed that CXCL13 is present in the CSF of patients with early acute LNB. Hence, it has also been proposed as a specific diagnostic marker for this condition. High concentrations of CXCL13 chemokine can be detected in the CSF several days before anti-Borrelia burgdorferi (Bb) antibodies occur [3].

Current studies have revealed that the CSF CXCL13 chemokine levels in patients with LNB correlate with pleocytosis and decrease after the initiation of antibiotic treatment [6, 7].

The intrathecal synthesis of anti-Bb antibodies may persist for years after a completed antibiotic treatment, but CXCL13 concentrations decrease rapidly after aetiological treatment. Thus, this marker is useful for monitoring response to treatment because it can draw a distinction between early and late infection. It was observed that in the case of LNB, CSF CXCL13 chemokine is a marker that shows the duration of the disease rather than its activity [8].

Literature suggests that CXCL13 and other chemotactic cytokines have a role in neuroinflammatory diseases. To date, most chemokines have been studied in small groups of patients, often focusing on one specific neuroinflammatory condition.

Studies have revealed high levels of CXCL13 in CSF, which often exceed serum levels and a low rate of protein transfer through the blood-brain barrier (BBB) in the CSF (<1%). It is assumed that the majority of CSF cytokines/chemokines are produced in the CNS of patients with an intact BBB.

Researchers observed significant correlations of cytokines/chemokines in patients with impaired BBB, which suggests that they are regulated in parallel in patients with neuroinflammation [9, 10].

The **aim of** our **study** was to analyse the levels of CXCL13 chemokine in the CSF and plasma in patients suffering from acute neurological diseases of infectious and non-infectious origin. The correlations of CXCL13 levels, pleocytosis intensity, and CSF biochemical parameters have also been analysed.

## Material and method

We conducted a prospective study in patients admitted to the 1st Infectious Diseases Clinic and 1st Clinic of Neurology, Tîrgu Mureș, Mureș County, Romania, during January 2015 - December 2015.

Group 1 comprised 38 patients (52.8%) *hospitalised with acute neuroinflammatory infections* (ANII), complaining of fever, intracranial hypertension syndrome (headaches, vomiting, photophobia), meningeal irritation (neck stiffness, positive Kerning's sign) and signs of brain damage (drowsiness, dizziness, temporospatial disorientation). On admission, these patients were diagnosed with: acute viral meningitis, unspecified aetiology - 16 patients, acute viral meningoencephalitis, unspecified aetiology - 10 patients, viral encephalomyelitis, unspecified - 3 patients, acute bacterial meningitis (various aetiologies) - 5 patients. The first group included: cases of acute meningoencephalitis with *Mycobacterium tuberculosis* (2 cases) and *Cryptococcus neoformans* (2 cases) in patients infected with human immunodeficiency virus (HIV), in an advanced stage of the disease, AIDS C3. The antiretroviral therapy of HIV patients

had been interrupted because of lack of adherence one year before admission.

Group 2 included 34 patients (47.2%) with *non-infectious neurological diseases* (NIND). These patients had normal body temperature on admission, but complained of severe headache (11 cases), unilateral facial paralysis (9 cases), hypoesthesia of unspecified causes (5 cases), transient cerebral ischemic stroke (3 cases), seizures (2 cases), hydrocephalus (2 cases), flaccid paralysis (1 case), tetraparesis (1 case).

None of the patients included in the study underwent antibiotic, corticosteroid or immunomodulator therapy before lumbar puncture.

This study was approved by the Research Ethics Committee of the University of Medicine and Pharmacy of Tîrgu Mureș and every patient signed an informed consent.

We collected CSF and serum from each patient included in the study. CSF cell count was performed immediately after sampling by using the Fuchs-Rosenthal Counting Chamber (ROTH Karlsruhe Germany). A number > 5 cells/mm$^3$ was considered pathological.

The level of CXCL13 chemokine in CSF and serum was determined using the Human CXCL13/ BLC/ BCA-1-Quantikine ELISA kit (R & D Systems, Minneapolis, MN USA). The analysis was carried out using the enzyme-linked immunosorbent assay (ELISA) technique, with monoclonal antibodies specific for BLC (B-Lymphocyte Chemoattractant)/ BCA-1 (B Cell-Attracting chemokine 1), or CXCL13, which had been pre-coated onto a microplate. The different levels were expressed in pg/mL, and the detection limit ranged from 0.43-3.97 pg/mL. All measurements were carried out according to the manufacturer's instructions.

Glucose concentrations in CSF were determined using the enzymatic method with hexokinase. Expected values in CSF were 40-70 mg/dL. The total protein concentration in the serum was determined by colorimetric assay (biuret reaction). Normal values in adults range between: 66-87 g/L. The turbidimetric method was used to determine CSF protein concentrations. Normal values range between: 150-450 mg/L. We used the Roche/Hitachi Cobas analyser for all measurements.

Serological investigations were carried out to confirm the presence of an infection with spirochete *Borrelia burgdorferi* (Bb.). We used the ELISA technique (Enzyme immunoassay for measurement of IgM/ IgG antibodies against the 14 kDa and OspC antigens of *Borrelia burgdorferi* in human serum, plasma and CSF, Kit Borrelia dreams InstrumentsGmbH DRG, Germany) to determine the presence of anti-Bb antibodies (IgM, IgG) both in CSF and serum. IgM, IgG antibodies > 11 U/mL were considered positive, between 9-11 U/mL borderline and <9 U negative. All patients were tested for syphilis by using hemagglutination assay, *Treponema pallidum* Hemagglutination assay (TPHA) with a reference range for antibody titer: <1/80 negative and > 1/80 positive.

**Statistical analysis** was performed using GraphPad InStat 3 and MedCalc Software, Version 12.5.0.0. Data were considered as nominal or quantitative variables. Nominal variables were characterised by using frequencies. Quantitative variables were assessed for the normality of distribution using the Kolmogorov-Smirnov test characterised by median distributions and IQR expressed as 25$^{th}$ and 75$^{th}$ percentile or by mean and standard deviation (SD), as appropriate. A chi-square test was used to compare the frequencies of nominal variables. The quantitative variables were compared using the Student test or Mann-Whitney test, as appropriate. The correlation between quantitative variables was assessed using Spearman's rho. We used the linear multivariate regression test and estimated the cumulative effect of the studied

predictor factors (age, CSF pleocytosis, glucose and proteins) for CXCL13 CSF levels in group 1 and group 2, respectively. The level of statistical significance was set at p<0.05.

## Results

This study comprised a total number of 72 patients of which 41 were males (56.94%) and 34 females (43.06%). Of the total studied patients, 37 (51.38%) came from urban areas and 35 (48.62%) from rural areas. The median age in group 1 (*infectious neurological disorder*) was 35 (25-54.5) years, and 33 (30-58.2) years in group 2 (*non-infectious neurological*), without any statistically significant difference (p-0.06). Demographic parameters showed no statistically significant differences in the two groups: gender (p-0.056) and provenance (p-0.47).

The median cellular elements in the cerebrospinal fluid of patients comprised in our study was 4 cells/mm$^3$ (3-54.75 cells/mm$^3$). CSF pleocytosis was higher (>5 cells/mm$^3$) in the ANII group (23 patients, 74.19% out of 31) than in the NIND group (7 patients, 22.5% out of 31), statistically significant difference (p-0.0001). The median value of CXCL 13 chemokine concentration in the CSF was 20.18 pg/mL (4.75-71.25) and 67.8 pg/mL (10.0-118.6) in plasma.

On the other hand, the median value of protein concentration in the CSF of group ANII was 45.5 mg/L (27.7-77.85), while being as high as 413 mg/L (192.0-725.5) (p-0.001; U test:189.5) in group NIND. No statistically significant differences were noted in the median values of plasma proteins in the two groups of patients: 6.4 g/dL (6.24-7.56) in the first group, and 6.99 g/dL (6.40-7.54) in the second one (p-0.83; U test: 502.5).

The median value of CSF glucose concentration in the ANII group was 62.5 mg/dL (52.7-70.25) and 63.0 mg/dL (59.1-74.6) (p-0.19; U test: 491.5) in group NIND. Anti Bb (IgM, IgG) antibodies in CSF and serum, as well as syphilis in plasma, tested negative **(Table 1)**.

Results of p<0.02 were subjected to a multivariate regression analysis, separately for the two groups.

The results of the multivariate regression analysis showed that the independent variables: CSF pleocytosis, glucose and proteins had a significant cumulative effect on CXCL 13 levels in the CSF in the ANII group but not in the NIND group. According to the unstandardized regression coefficients in group 1, the CSF pleocytosis and protein were positively associated (p 0.05, p 0.001 respectively), but the CSF glucose was negatively associated (p-0.039) with the CXCL13 levels in the CSF, **(Table 2)**.

We analysed the correlation between CSF CXCL13 concentrations and pleocytosis in the 72 patients and found a positive statistically significant association (p-0.02, Spearman's coefficient of rank correlation (rho) =0.39), **(Figure 1)**.

CXCL13 levels in the CSF of the ANII group had a median value of 23.07 pg/mL (4.46-145.9) and significantly different in group NIND: 11.51 pg/mL (5.11-67.8) (p-0.03; U test: 463) – **Figure 2a**.

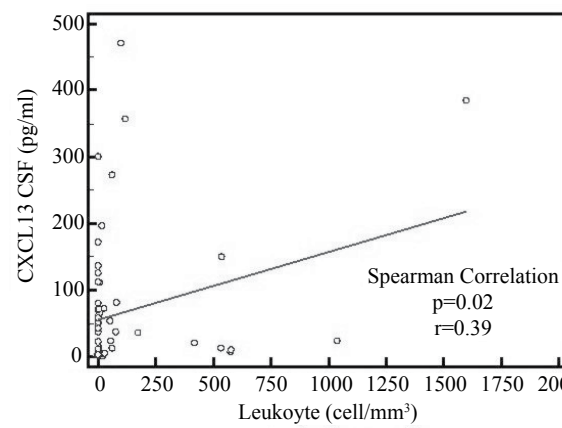


**Figure 1. Correlation between CSF CXCL13 and pleocytosis**

**Table 1. Demographic, serologic and biochemical data in the studied groups**

| Group criteria | Patients | ANII 52.8% (38) | NIND 47.2% (34) | P value |
|---|---|---|---|---|
| **Age (years)** | | 35 (25-54.5) | 33 (30-58.2) | 0.06* |
| **Male/Female** | 41/31 | 26/12 | 15/19 | 0.056** |
| **Provenience Urban/Rural** | 37/35 | 18/20 | 19/15 | 0.47** |
| **CSF Pleocytosis** | | | | **0.0001**** |
| **>5 cell/mm$^3$** | 4.0 (3-54.75) | 23 | 7 | |
| **<5 cell/mm$^3$** | | 8 | 24 | |
| **CSF CXCL 13 (pg/mL)** | 20.18 (4.75-71.25) | 23.07 (4.46-145.9) | 11.51 (5.11-67.8) | **0.03*** |
| **Serum CXCL 13 (pg/mL)** | 67.8 (10.0-118.6) | 108.1 (35.0-274.8) | 50.7 (9.0-81.4) | **0.001*** |
| **CSF Protein (mg/L)** | 79 (37.45-414.5) | 45.5 (27.7-77.85) | 413.0 (192.0-725.5) | **0.001*** |
| **Plasma Protein (g/dL)** | 6.99 (6.47-7.54) | 6.4 (6.24-7.56) | 6.99 (6.40-7.54) | 0.83* |
| **CSF Glucose (mg/dL)** | 63 (55.0-71.5) | 62.5 (52.7-70.25) | 63.0 (59.1-74.6) | 0.19* |
| **Anti-Bb Antibody IgM, IgG – CSF** | | | | |
| **>11 U/L positive** | | 0 | 0 | |
| **<9 U/L negative** | | 38 | 34 | |
| **Anti-Bb Antibody IgM, IgG – serum** | | | | |
| **>11 U/L positive** | | 0 | 0 | |
| **<9 U/L negative** | | 38 | 34 | |
| **TPHA – serum** | | | | |
| **<1/80 negative** | | 38 | 34 | |
| **>1/80 positive** | | 0 | 0 | |

**Mann Whitney test, Data expressed by median (IQR) **Chi square test, Data expressed by no.*

**Table 2. Results from liniar multivariate regression**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| ANII group | Age (years) | 0.215 | 1.267 | 0.027 | 0.170 | 0.86 |
| | CSF Pleocytosis | 0.039 | 0.058 | 0.110 | 0.683 | **0.05** |
| | CSF Glucose (mg/dL) | -1.389 | 0.635 | -0.348 | -2.188 | **0.039** |
| | CSF Protein (mg/L) | 0.370 | 0.101 | 0.572 | 3.656 | **0.001** |
| NIND group | Age (years) | -0.436 | 0.660 | -0.136 | -0.661 | 0.51 |
| | CSF Pleocytosis | 0.354 | 0.444 | 0.168 | 0.797 | 0.43 |
| | CSF Glucose (mg/dL) | 0.274 | 0.262 | 0.216 | 1.046 | 0.30 |
| | CSF Protein (mg/L) | 0.766 | 0.014 | 0.0001 | 0.001 | 0.95 |
| Dependent Variable: CSF CXCL13 (pg/ml) | | | | | | |

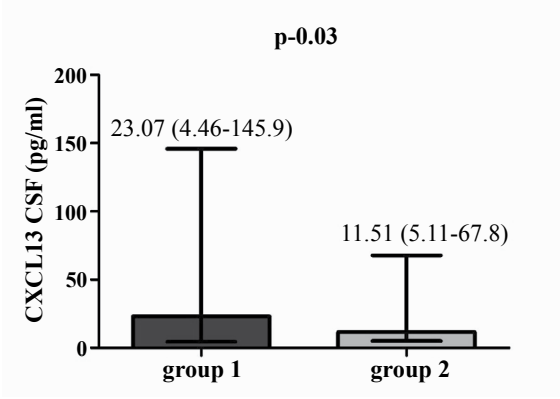


**Figure 2a. CSF CXCL13 comparison between the two groups**

*(Group 1: Infectious neurological disorder; Group 2: Non-infectious neurological)*

*Data expressed by median (IQR)*

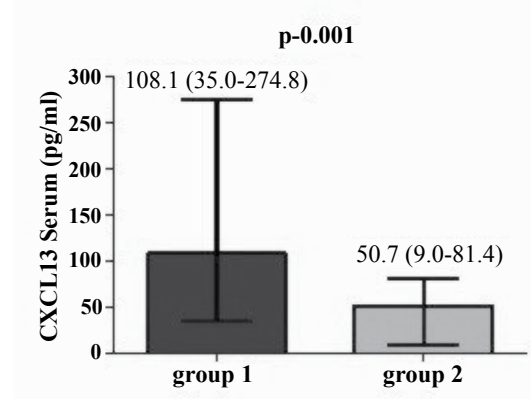


**Figure 2b. Serum CXCL13 comparison between the two groups**

*(Group 1: Infectious neurological disorder; Group 2: Non-infectious neurological)*

*Data expressed by median (IQR)*

In the ANII group of patients CXCL13 concentrations in plasma presented a median value of 108.1 pg/mL (35.0-274.8), but lower in the NIND group with a median value of 50.7 pg/mL (9.0-81.4), (p-0.001; U test: 345.5) – **Figure 2b**.

## Discussions

CXCL13 chemokine (BCA-1 B cell-attracting chemokine 1) is known to influence B cell migration. This chemokine is preferentially expressed in lymphoid organs and regulates the migration and compartmentalization of lymphocytes and antigen presenting cells. CXCL13 is present in the CSF and CNS, especially during neuroinflammation [1,11].

During this study, we monitored the CXCL13 levels in CSF and plasma of patients suffering from acute infectious neurological diseases (acute viral/bacterial meningitis, acute meningoencephalitis, encephalomyelitis) and compared them to non-infectious neurological diseases (severe headache, unilateral facial paralysis, hypesthesia of unspecified causes, transient cerebral ischemic stroke, seizures, hydrocephalus, flaccid paralysis and tetraplegia).

CXCL13 chemokine was correlated with the basic parameters of CSF (pleocytosis, protein and glucose levels) in patients with infectious and non-infectious neurological disorders.

CFS pleocytosis was significantly higher in the ANII group than in group NIND (p-0.0001). Since patients with acute viral/ bacterial meningitis were included in the first group, this result was somehow expected. Cellular elements, leucocytes in the ANII group had higher values (between 10-25000 cell/mm$^3$) in comparison with NIND group of patients with non-inflammatory neurological diseases (between 1-40 cell/mm$^3$).

The median value of CSF CXCL13 concentrations in the 72 studied patients was 20.18 pg/mL, while the median value of plasma level was three times higher 67.8 pg/mL.

CSF CXCL13 median values were elevated in patients with neuroinfectious diseases compared to patients with non-infectious neurological disorders (p-0.03). The highest levels of CSF CXCL13 concentrations, with values between 250-470 pg/mL were observed in the ANII group: five cases of acute bacterial meningitis, three with acute encephalomyelitis, two with

tuberculous meningitis (HIV patients), and two with Cryptococcus neoformans (HIV patients). The four HIV patients suffered from stage C3 AIDS: CD4 cell count <200/μL and viral load (HIV RNA)>100000 copies/mL. Antiretroviral therapy had been discontinued about a year prior to admission. Other studies also published high concentrations of CXCL13 in patients with viral/ bacterial meningitis, encephalitis, medullary syndrome, hypogammaglobulinaemia, meningeal tuberculosis, and autoimmune diseases [8,12,13]. No study has revealed high levels of CSF CXCL13 in patients with HIV who do not have acute intrathecal inflammatory changes, indicating that chemokine in these patients increases only in the presence of an associated neuroinfection and not in chronic HIV infection.

CSF CXCL13 concentrations can be particularly useful when diagnosing Neurosyphilis in HIV-infected patients because it is independent of pleocytosis and HIV disease markers. CSF CXCL13 and plasma concentrations can be used to confirm or refute the diagnosis of neurosyphilis when the Venereal Disease Research Laboratory (VDRL) test in the CSF is nonreactive, but pleocytosis is high [14-17]. In our NIND group only one patient with transient cerebral ischemic stroke presented a high value of CXCL13 concentration 171.5 pg/mL.

CXCL13 chemokine has been mentioned as a diagnostic parameter with increased specificity for LNB, and as a marker for the evolution of the diseases. Neurosyphilis and LNB share similar characteristics. Studies report that patients with neurosyphilis and LNB showed significantly higher levels of CXCL13 chemokine than patients with other inflammatory neurological diseases, such as MS [18,19], compared with other diseases such as neurosyphilis, where studies reported much higher CSF CXCL13 concentrations, with values up to 37.000 pg/mL and LNB values between 424-158.000 pg/mL [8, 20].

In our study, we presented values of CSF CXCL13 concentrations. We had no patients with anti-Bb antibodies. IgM, IgG, and syphilitic infection were not confirmed, so we did not expect to have high values for CXCL13. According to some studies, the presence of high concentrations of CXCL13 in the CSF is not completely specific to LNB [18,21]. Other researchers specify that high levels of CXCL13 are not exclusively emphasised in case of LNB, but also in other infectious diseases of the CNS. In the present study, CSF CXCL13 levels did not exceed plasma levels. The plasma concentration of CXCL13 in the ANII group showed a median value of 108.1 pg/mL, which is twice as high as in the group of patients with non-infectious neurological diseases, with a median value of 50.7 (p- 0.001).

The early release of CXCL13 is supported by experimental findings which assert that CXCL13 can systematically activate and attract B cells to CNS and can create a mechanism that crosses the BBB. Chemokine CXCL13 is mainly produced in the follicular dendritic cells (FDCs) of lymphoid tissues. However, FDC exists in CSF, so that an early release of CXCL13 in CSF is possible. During systemic inflammation, chemokines can be selectively induced in several cells, including microglia, astrocytes, neurones, and endothelial cells [18, 22].

Interestingly, the link between CXCL13 in CSF and neurological signs has been recently emphasised. CXCL13 and/or IL-10 can promote lymphoid tissue neogenesis, which is an associated pathogenic factor with inflammatory infiltrations in rheumatoid arthritis or the demyelination of the white matter in case of neuroinflammatory diseases. Polymerase chain reaction techniques demonstrated that CXCL13 is produced in case of active demyelinating diseases, and not in inactive or chronic lesions within the CNS of patients who have never had neuro-

logical diseases. Therefore, CXCL13 is one of the factors that attract and arrest B and T cells, which play a role in the inflammatory processes of neuroinflammatory disorders [1, 3, 6, 19]. We did not include in our study patients suffering from demyelinating neurological diseases.

CXCL13 was constantly increased in patients with neurological infectious diseases. It was also present in the group of patients with non-infectious neurological diseases, but with much lower values. Thus, research reports mention that CXCL13 is involved in the functioning of CSF in psychopathological conditions, which seems to play a role as neuromodulator by regulating the release of neurotransmitters or by modulating the activity of ion channels both at the presynaptic and postsynaptic level [2, 18, 23].

The median value of proteins in the CSF of patients belonging to the ANII group was 45.5 mg/L, which was lower than in the second group of patients, 413.0 mg/L (p-0.001), probably due to the higher mean age of patients with non-infectious neurological diseases. In our study, we found no significant correlations between relevant CXCL13 levels in CSF and plasma, age, gender, CSF glucose, and protein levels. According to the unstandardized coefficients of linear multivariate regression in the ANII group, with acute infectious neurological diseases, we found that the level of CXCL13 chemokines in CSF positively associated with the pleocytosis and CSF proteins (p-0.05, p-0.001) and at the same time negatively with the level of glucose in CSF (p-0.039).

Previous studies have also highlighted the fact that patients with encephalitic and myelitic syndrome have higher levels of CXCL13 and CSF proteins, which proves that this chemokine is not evidenced solely in LNB, neurosyphilis and MS, but also in other neurological infectious diseases as an inflammatory marker of CNS [1, 6].

Performing the correlation between CSF CXCL13 concentrations and pleocytosis we observed that the higher pleocytosis level, the higher concentration of CXCL13 were obtained, which generated a statistically significant difference (p-0.02). In a previous study, a significant correlation was found between mononuclear cells and CSF CXCL13 chemokine. The reason for this correlation is still unknown [3].

We observed a statistically significant association (p-0.001) between the serum levels of a CXCL13 chemokine of the two groups of patients. It is important to notice that the median value in the ANII group was more than double (108.1 pg/mL) than in the NIND group (50.70 pg/mL).

Studies mention that CXCL13 seems to play an important role in the recruitment of plasma cells. In patients with intact BBB, chemokine CXCL13 correlates with the number of cells in the CSF of patients [5].

The origin of cytokines/ chemokines found in CSF is uncertain. They could be produced locally in the CNS or originate in the peripheral nervous system (PNS), especially in patients with BBB impairment [7, 8, 23].

Given the high level of CSF CXCL13 chemokine, which sometimes exceeds the serum level and the low rate of blood protein diffusion in the CSF through BBB, it is assumed that at least in patients with an intact BBB, most chemokines/ cytokines in the CSF are produced within the CNS. A significant correlation of CXCL13 chemokine in patients with impaired BBB suggests that this is regulated in parallel in patients with neurological infectious diseases [24-26].

**Conclusions:** CXCL13 levels were significantly increased in patients with acute infectious neurological diseases compared to non-infectious diseases. Our study found that CXCL13 chemokine concentration was significantly correlated with the number of cells and proteins in the CSF of patients suffering from neuroinfectious.

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## Conflict of interest disclosure

None of the authors has any conflict of interest to declare.

## Abbreviations

| | |
|---|---|
| AIDS | = Acquired immune deficiency syndrome |
| ANII | = Acute neuroinflammatory infections |
| Bb. | = *Borrelia burgdorferi* |
| BBB | = Blood-brain barrier |
| BCA- 1 | = B Cell – Attracting Chemokine 1 |
| BLC | = B – Lymphocyte Chemoattractant |
| CSF | = Cerebrospinal fluid |
| HIV | = Human immunodeficiency virus |
| LNB | = Lyme neuroborreliosis |
| NIND | = Non-infectious neurological diseases |
| TPHA | = *Treponema Pallidum* Haemagglutination Assay |
| VDRL | = Venereal Disease Research Laboratory Test |

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