Use of cerebrospinal fluid CXCL13 concentration for diagnosis and monitoring of neurosyphilis: a three-case report

Utilização da concentração de CXCL13 no líquido cefalorraquidiano para o diagnóstico e monitoramento de neurossífilis: um relato de três casos

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ABSTRACT

Introduction: Previous retrospective studies have demonstrated that the concentration of chemokine ligand CXCL13 in cerebrospinal fluid (CSF-CXCL13) is a promising biomarker in the diagnosis of neurosyphilis and, additionally, in the monitoring of therapeutic efficacy. **Objective:** To describe three cases of patients with neurosyphilis (NS) treated at Hospital Universitário Gaffrée e Guinle, in Rio de Janeiro, Brazil, with suspected active syphilis with neurological symptoms. **Case report:** Three patients from Rio de Janeiro, Brazil, were investigated for symptomatic NS. The concentration of CSF-CXCL13 was prospectively performed by enzyme-linked immunosorbent assay (ELISA) in all participants at baseline and in follow-up visits at 3 months after therapy. CSF-CXCL13 concentrations were significantly higher in all three patients with established NS. The CSF-CXCL13 concentrations decreased after 3 months of therapy compared to baseline in all cases reported. The added high concentration of CSF-CXCL13 plus CSF-TPHA reactivity above 1:40 titer agreed with the diagnosis of NS in 100% of the cases. **Conclusion:** In this case series, we present three cases of NS diagnosed using CXCL13 in CSF as a complementary test. These case series suggest that the clinical use of CSF-CXCL13 is useful as a supplementary biomarker for NS and for monitoring the effectiveness of NS therapy, especially in patients with nonreactive CSF-VDRL, excluding other neurologic diseases.

Keywords: CXCL13. Neurosyphilis. Cerebrospinal fluid. *Treponema pallidum*.

RESUMO

Introdução: Estudos retrospectivos anteriores demonstraram que a concentração do ligante de quimiocina CXCL13 no líquido cefalorraquidiano (CSF-CXCL13) é um biomarcador promissor no diagnóstico de neurossífilis e, adicionalmente, no monitoramento da eficácia terapêutica. **Objetivo:** Descrever três casos de pacientes com neurossífilis (NS) tratados no Hospital Universitário Gaffrée e Guinle no Rio de Janeiro, Brasil, com suspeita de sífilis ativa com sintomas neurológicos. **Relato de casos:** Três pacientes do Rio de Janeiro, Brasil, foram investigados para NS sintomática. A concentração de CSF-CXCL13 foi prospectivamente realizada por ensaio imunoenzimático (ELISA) em todos os participantes no início e nas visitas de acompanhamento três meses após a terapia. As concentrações de CSF-CXCL13 foram significativamente mais altas em todos os três pacientes com NS estabelecida. As concentrações de CSF-CXCL13 diminuíram após três meses de terapia em comparação com os níveis iniciais em todos os casos relatados. A alta concentração adicional de CSF-CXCL13 combinada com a reatividade CSF-TPHA acima do título 1:40 foram condizentes com o diagnóstico de NS em 100% dos casos. **Conclusão:** Nesta série de casos, apresentamos três casos de neurossífilis diagnosticados usando CXCL13 no LCR como teste complementar. Essas séries de casos sugerem que o uso clínico de CSF-CXCL13 é útil como biomarcador suplementar para NS e como monitoramento da eficácia da terapia NS, especialmente em pacientes com CSF-VDRL não reativo, excluindo outras doenças neurológicas.

Palavras-chave: CXCL13. Neurossífilis. Líquido cefalorraquidiano. *Treponema pallidum*.

INTRODUCTION

Syphilis is an infectious disease caused by the spirochete bacteria *Treponema pallidum* subspecies *pallidum* (*T. pallidum*). It has been called the "great imitator" due to the multitude of symptoms it produces. The diagnosis becomes further muddled by periods of active disease and latency. Regardless primary, secondary, and tertiary syphilis, the *T. pallidum* can be able to affect the Central Nervous System (CNS) at any time after infection. While most patients can mount an immune response that effectively clears CNS invasion without long-term complications, a minority go on to develop neurologic involvement. Additionally, the belief is that bacterial neuroinvasion occurs in all patients, and it is a failure of clearance, which results in the condition[1,2].

OBJECTIVE

To demonstrate the concentration of chemokine ligand CXCL13 in cerebrospinal fluid (CSF-CXCL13) through a detailed description of three distinct NS cases treated at Hospital Universitário Gaffrée e Guinle, in Rio de Janeiro.

CASE REPORT

Between March 2018 and November 2020, three patients went to Hospital Universitário Gaffrée e Guinle, in Rio de Janeiro, Brazil, with suspected active syphilis with neurologic symptoms. The referral to our institution for potential NS was motivated by clinical indications of potential neurological or ophthalmologic involvement, as adjudged by the primary referring healthcare provider. According to the Brazilian

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diagnostic protocol, the confirmation of symptomatic NS requires designated clinical, serological, and CSF abnormalities criteria[3]. Conversely, the occurrence of a nonreactive CSF Nontreponemal Test (NTT) in conjunction with nonspecific CSF irregularities in patients under strong suspicion of NS introduces substantial diagnostic challenges.

Neurosyphilis represents a severe sequela of untreated syphilis, affecting the central nervous system (CNS) and precipitating an array of neurological manifestations. The challenges in its diagnosis arise from the non-specificity of serological examinations and the diverse clinical presentations. The chemokine, CXCL13 — a B cell chemoattractant — has previously been detected at elevated concentrations in the CSF of patients diagnosed with neuroborreliosis[4], Consequently, it has undergone investigation as a supplementary diagnostic test for NS[5-10]. Within the context of this report, we delineate three distinct NS cases from local population, wherein the CSF concentrations of CXCL13 (EUROIMMUN AG, Lübeck, Germany) were employed as ancillary diagnostic tools. In each instance, the CSF-CXCL13 concentrations were found augmented, facilitating the diagnosis of NS, and subsequently evidencing a decline in post-therapeutic intervention. These instances underscore the potential diagnostic efficacy of CSF-CXCL13 in the context of NS:

Case #1: A 57-year-old man initially complained of pain and an increase in abdominal volume that had been present for two months. His symptoms worsened and he developed constipation, urinary incontinence, paraparesis, and numbness in his lower limbs. Upon admission, he showed postural instability, difficulty walking, and reduced strength in both legs. A magnetic resonance image (MRI) of his spine revealed extensive longitudinal transverse myelitis with multiple nodular lesions. Analysis of his CSF showed an elevated number of 80 WBC/μL, but protein levels were normal at 32 mg/dL. The patient's serum FTA-ABS test was reactive, and his serum VDRL test was reactive at a titer of 1:128, but CSF-VDRL was nonreactive. The CSF-TPHA test was reactive at a titer of 1:40. Additionally, the CSF-CXCL13 was markedly elevated at 895 pg/mL. The patient was diagnosed with symptomatic NS (transverse myelitis and primary optic atrophy) and was treated with intravenous Benzylpenicillin potassium (crystalline) for 14 days. After three months, the patient showed improvement in neurological symptoms, and his CSF-CXCL13 decreased significantly to 100.2 pg/mL, and his CSF-TPHA titers also decreased to 1:5, and the CSF cellularity returned to normal.

Case #2: A 60-year-old male patient, presented with a 3-year history of slowly progressive weakness of both lower limbs. Over the next few months he developed visual dysfunction, which was followed by increase in his weakness in a way that he had to rely on the support of a wheelchair. Fundus examination revealed pallor of the optic disc in both eyes and the discs appeared chalky white on both sides, which was suggestive of primary optic atrophy. An MRI of brain and spinal cord was normal. The CSF analysis showed mild pleocytosis at 5 WBC/μL, normal protein level at 39 mg/dL, but elevated anti-treponemal antibody index at 2.7 (ref. £1.5). The patient's serum VDRL was reactive at a titer of 1:128, and CSF VDRL was reactive at a titer of 1:2. CSF FTA-ABS was reactive, and CSF-TPHA was reactive at a titer of 1:320. CSF CXCL13 was elevated at 290.4 pg/mL. The patient was diagnosed with symptomatic NS (acute transverse myelitis) and treated with intravenous Benzylpenicillin potassium (crystalline) for 14 days. After three months, the patient showed improvement in neurological symptoms and markedly decreased CSF-CXCL13 to 118.9 pg/mL and CSF-TPHA titers to 1:160 with CSF cellularity and protein normalization.

Case #3: A 53-year-old male presented with mental confusion and behavior change two months after being diagnosed with stroke with sudden onset aphasia and hemiplegia. A new MRI revealed multiple foci and discrete areas with hyperintensity on T2 and FLAIR in the brain, and EEG showed globally hypovoltage EEG tracing compatible with diffuse cortical damage. The CSF analysis showed no pleocytosis at 2 WBC/μL and markedly elevated CSF protein at 78.5 mg/dL. The patient's serum VDRL was reactive at a titer of 1:64, CSF-VDRL was reactive at a titer of 1:1, and CSF-TPHA was reactive at a titer of 1:2560. The CSF-CXCL13 was markedly elevated at 14,440 pg/mL. The patient was diagnosed with symptomatic NS (acute meningoencephalitis) and received intravenous ceftriaxone treatment after a penicillin reaction with seizure. After three months, the patient showed improvement in neurological symptoms and markedly decreased CSF-CXCL13 to 118.9 pg/mL and CSF-TPHA titers to 1:640 with CSF cellularity and protein normalization.

All patients were tested negative for HIV infection, and a confirmative PCR test for *Treponema pallidum* subsp. *Pallidum* (*tpp*15) was performed in all cases to confirm the diagnosis of NS (**Table 1**).

Table 1. Clinical and Laboratory Features of the NS cases.

Case	Age	Sex	Clinical presentation	CSF WBC cells/μL	CSF protein mg/mL (ref. <40)	Serum VDRL	CSF VDRL	CSF-FTA-ABS	CSF-PCR for T. pallidum (tpp15)	Qalb	QigG Trep (ref. <1,5)	CSF TPHA baseline	CSF TPHA 3 months	CSF-CXCL13 (pg/mL) baseline	CSF-CXCL13 (pg/mL) 3 months
#1	57	M	Menigovascular Medulary	80	103.0	1:32	NR	NR	Positive	23.0*	1.3	1:40	1:5	895.0	49.8
#2	60	M	Menigovascular Medulary	5	39.0	1:128	1:2	NR	Positive	6.0	2.7**	1:320	1:160	290.4	100.2
#3	53	M	Menigovascular Cerebral	2	78.5	1:64	1:1	Reactive	Positive	7.1	1.2	1:2560	1:640	14,443	118.9

Abbreviations: Qalb: CSF/serum albumin coefficient; NR: Non-Reactivity;); Ref.: Reference value; TT: Treponemal Test; AI: Anti-*Treponema pallidum* IgG antibody index. *BBB dysfuntion; †Values ≥1.5 indicates *T. pallidum* specific IgG intrathecal production.

DISCUSSION

The diagnosis of NS can be challenging due to the lack of specificity of serological tests and the variable clinical presentation. Regardless of whether it is primary, secondary, and tertiary syphilis, the *T. pallidum* can affect the CNS at any time after infection. While most patients can mount an immune response that effectively clears CNS invasion without long-term complications, a minority go on to develop neurologic involvement. Neurosyphilis has been divided into early and late stages. The early stages include asymptomatic meningitis, symptomatic meningitis, and meningovascular syphilis, while the late stages include dementia paralytic and *tabes dorsalis*. Ophthalmic and otologic syphilis can occur at any time, but often accompany the acute meningitis of early NS[11]. Furthermore, unlike traditional stroke syndromes, NS can present without vascular risk factors and at a young age[12-14].

The CSF-VDRL is considered the "gold standard" for diagnosis of NS but has low sensitivity (from 30 to 70%)[13,14]. In our series, there were three NS cases, of which two had reactive CSF-VDRL (66%). In addition to the aforementioned diagnostic methods, several other biomarkers have been proposed in the literature as potential tools for the diagnosis of NS. These include the utilization of the anti-*Treponema pallidum* Hemagglutination Assay (TPHA) in CSF, the anti-treponemal antibody index, as well as the polymerase chain reaction (PCR) specifically targeting the *T. pallidum* by *tpp*15 gene[15-17]. However, only CSF-TPHA in titers ³1:40 performed better (100%) than CSF-VDRL in our cases, but like *Treponema pallidum* Particle Agglutination Assay (TPPA) in lower titers (<1:640) it is considered poor in specificity[16].

Since 2010, various studies have demonstrated that CSF concentration of the C-X-C motif chemokine ligand 13, commonly referred to as CXCL13, is significantly elevated in patients with NS, irrespective of the presence of CSF pleocytosis or HIV infection markers. Therefore, CSF-CXCL13 concentration has emerged as a promising biomarker for diagnosing NS and evaluating therapeutic efficacy in both HIV-positive and negative patients[5]. The CXCL13 is a B-cell chemoattractant chemokine and has been observed to be significantly elevated in CSF samples from patients with untreated NS. This elevation is likely a result of aberrant humoral immune responses, potentially through the formation of Ectopic Germinal Centers (EGC). Such findings indicate a possible molecular mechanism responsible for neurological damage in NS, particularly in symptomatic cases. This not only underscores the importance of the molecule's value in understanding the condition but also emphasizes the potential necessity to reassess current clinical practices or diagnostic/prognostic methodologies[18].

In a comprehensive investigation by Dersch et al., evidence was presented suggesting a notable decline in CSF-CXCL13 levels among patients diagnosed with NS upon the administration of suitable antibiotic therapy. This descending trend was concomitantly associated with an amelioration of clinical manifestations and a diminished WBC count within the CSF. Thus, CSF-CXCL13 may be proposed as a supplementary indicator of CNS inflammatory processes[6].

In research conducted by Kojima et al., it was determined that the amalgamation of CXCL13 and the anti-treponemal antibody index exhibited a sensitivity of 100% and a specificity of 98% in diagnosing NS[19]. Nonetheless, to validate these findings and ascertain the most efficacious diagnostic algorithm for NS, more comprehensive studies are imperative. It should be noted that all existing studies on this subject are retrospective in nature, and none have incorporated the CSF-CXCL13 concentration into treatment decision-making.

Our collective case analysis underscores the potential efficacy of CXCL13 as an auxiliary diagnostic measure for NS, especially among individuals with inconclusive cerebrospinal fluid tests or ambiguous neurological manifestations. It further emphasizes the paramount significance of incorporating this test into the diagnostic framework for patients with a presumptive diagnosis of NS. It is imperative for medical practitioners to be cognizant of the potential diagnostic role of CSF-CXCL13 in NS cases. Further empirical investigations are requisite to delineate its diagnostic precision, cut-off level, and applicability in clinical settings.

Strengths

The cases report under discussion presents crucial insights into the challenges and exploratory solutions related to the diagnosis and management of NS based on three distinctive cases. The core strength of this report hinges upon its investigative approach toward the employment of CSF-CXCL13 concentrations as an auxiliary diagnostic and monitoring tool. Despite the diverse and, at times, insidious manifestations of NS across the cases, the elevated CSF-CXCL13 concentrations consistently emerged as a reliable indicator, not only facilitating the initial diagnosis but also assisting in gauging the efficacy of therapeutic interventions, as evidenced by the post-treatment concentration declines. Furthermore, the report robustly bridges empirical research and clinical practices by illustrating the practical application and potential viability of CSF-CXCL13 in real-world clinical scenarios, especially in instances where traditional diagnostic criteria are inconclusive or non-reactive, like non-reactive CSF-VDRL despite a strong clinical suspicion of NS. The contextualization of previous research within actual patient cases provides a tangible framework that could potentially guide future research, fostering a deeper understanding of NS, while also paving the way towards refining and augmenting existing diagnostic protocols by incorporating innovative biomarker approaches. The cases, in essence, underscore the potential of CSF-CXCL13 to not only bolster diagnostic confidence in ambiguous NS cases but also to serve as a valuable biomarker in tracking therapeutic response and efficacy. This may indeed lay down a pathway for future studies, aspiring to validate and establish the clinical applicability and reliability of CSF-CXCL13 in the diagnostic and management arsenal against NS, both as a definitive indicator and a tool to refine treatment strategies.

Limitations

Primarily, the small sample size of three patients inherently restricts the generalizability of the findings to the wider patient population. In addition, the investigation is also geographically

and demographically limited to one hospital in Rio de Janeiro, Brazil, raising questions about its applicability to diverse populations with potentially varying epidemiological and clinical characteristics. Moreover, the lack of a standardized or established threshold for CSF-CXCL13 concentrations to diagnose NS may cast doubts on the diagnostic specificity and sensitivity of the marker across different patient demographics and co-morbid conditions. Lastly, the report does not engage in a comparative analysis with a control group, which limits the ability to correlate CSF-CXCL13 concentrations exclusively to NS pathophysiology, hindering the establishment of causality or direct association. Consequently, while the case report undeniably presents a compelling argument for the potential use of CSF-CXCL13 in NS diagnosis and management, these limitations underscore a palpable need for further, more robust and systematic investigations to validate and optimize the clinical implementation of this promising biomarker.

CONCLUSION

Neurosyphilis remains an important diagnostic challenge due to its variable clinical presentation and the lack of specificity of serological tests. CXCL13 in CSF has emerged as a promising complementary test for the diagnosis of NS, and our case series highlights its utility in supporting the diagnosis of the disease. Clinicians should be aware of the potential role of CXCL13 in the diagnostic workup of patients with suspected NS, and further studies are needed to establish its diagnostic accuracy and utility in clinical practice.

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Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and all patients provided written informed consent for the use of their clinical data in this case series. The study was approved by the Ethical Research Committee of Hospital Universitário Gaffrée e Guinle by number CAAE 66558117.0.0000.5258. Patient identities have been protected in accordance with patient confidentiality regulations.

Participation of each author

RSC: Conceptualization, Metodology, Project administration, Writing – original draft. ICR: Data curation, Writing – review & editing. MMS: Formal analysis, Validation, visualization. FRAF: Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare no conflicts of interest.

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