North Clin Istanb 2023;10(3):341–344
doi: 10.14744/nci.2022.77861

Original Article NEUROSCIENCE

Serum CXCL5 as a biomarker in multiple sclerosis and neuromyelitis optica spectrum disorder

Zerrin Karaaslan,[1] Vuslat Yilmaz,[1] Hande Yuceer,[1] Elif Sanli,[1] Halil Ibrahim Akcay,[2] Murat Kurtuncu,[2] Recai Turkoglu,[3] Erdem Tuzun[1]

[1]Department of Neuroscience, Istanbul University, Aziz Sancar Institute of Experimental Medicine, Istanbul, Turkiye
[2]Department of Neurology, Istanbul University Istanbul Faculty of Medicine, Istanbul, Turkiye
[3]Department of Neurology, Haydarpasa Numune Training and Research Hospital, Istanbul, Turkiye

ABSTRACT

OBJECTIVE: Our aim was to determine whether serum C-X-C motif chemokine 5 (CXCL5) may serve as a diagnostic biomarker for relapsing-remitting multiple sclerosis (RRMS) as well as a marker that can be used to predict treatment response.

METHODS: CXCL5 levels were measured by ELISA in sera of 20 RRMS patients under fingolimod treatment, 10 neuromyelitis optica spectrum disorder (NMOSD) patients, 15 RRMS patients presenting predominantly with spinal cord and optic nerve attacks (MS-SCON), and 14 healthy controls.

RESULTS: Fingolimod treatment significantly reduced CXCL5 levels. CXCL5 levels were comparable among NMOSD and MS-SCON patients.

CONCLUSION: Fingolimod might regulate the innate immune system. Serum CXCL5 measurement does not differentiate between RRMS and NMOSD.

Keywords: C-X-C motif chemokine 5; fingolimod; multiple sclerosis; neuromyelitis optica spectrum disorder.

Cite this article as: *Karaaslan Z, Yilmaz V, Yuceer H, Sanli E, Akcay HI, Kurtuncu M, et al. Serum CXCL5 as a biomarker in multiple sclerosis and neuromyelitis optica spectrum disorder. North Clin Istanb 2023;10(3):341–344.*

Multiple sclerosis (MS) is the most common autoimmune demyelinating disease of the central nervous system. Although myelin-reactive CD4+ T cells are the main drivers of disease pathogenesis, innate immune system cells recruited to MS lesions by chemokines contribute to disease pathogenesis. Neutrophils migrate to the inflammation site as the first defenders of the innate immune system and they are detected in the cerebrospinal fluid (CSF) of MS patients during relapses [1].

C-X-C motif chemokine 5 (CXCL5), also known as epithelial neutrophil-activating peptide 78, is a potent chemokine that signals through C-X-C motif chemokine receptor 2 and attracts neutrophils to the lesion site [2]. CXCL5 levels are elevated during MS attacks and correlate with disease severity [3]. Suppression of neutrophils attenuates disease severity in experimental autoimmune encephalomyelitis [4]. Fingolimod treatment was shown to decrease CXCL5 levels in a demyelination model using cerebellar slice cultures, suggesting that besides prevention of T lymphocyte migration, regulation of the innate immune system could contribute to the mechanism of action of fingolimod [5].

Neutrophil aggregation is a typical feature of neuromyelitis optica spectrum disorder (NMOSD). CXCL5 level was found to be correlated with disease severity in NMOSD but not in MS in contrast with previous study [3],

Received: May 20, 2021 *Revised:* November 23, 2021 *Accepted:* May 30, 2022 *Online:* June 19, 2023

Correspondence: Erdem TUZUN, MD. Istanbul Universitesi Aziz Sancar Deneysel Tip Enstitusu, Sinirbilim Anabilim Dali, Istanbul, Turkiye.
Tel: +90 212 414 20 00 - 33321 e-mail: drerdem@yahoo.com

raised the question whether CXCL5 might serve differentiation of NMOSD from relapsing-remitting MS (RRMS) presenting predominantly with spinal cord and optic nerve attacks (MS-SCON) [6].

In this study, we first aimed to investigate the possible utility of CXCL5 as a biomarker for the differentiation of NMOSD from MS presenting with clinical features reminiscent of NMOSD. Furthermore, we investigated the role of CXCL5 as a biomarker for the prediction of treatment response in MS patients.

Highlight key points

- Fingolimod treatment significantly reduced CXCL5 levels.
- Regulation of the innate immune system could be one of the action mechanisms of fingolimod.
- CXCL5 levels do not reflect the severity of NMOSD and MS and do not provide a prediction about prognosis after fingolimod treatment.

MATERIALS AND METHODS

Subjects

Ten NMOSD (6 aquaporin-4 IgG seropositive) patients who fulfilled international diagnostic criteria for NMOSD [7], 15 MS-SCON patients, and 14 age/sex-matched healthy controls with no prior inflammatory or neurological disorder history were recruited (Table 1).

All patients were in remission and not under steroid treatment. Both NMOSD and MS-SCON patients were under immunomodulating treatment (azathioprine, interferon-beta, and glatiramer acetate). All MS-SCON patients fulfilled the diagnostic criteria of MS and none of them had aquaporin-4 IgG, long extensive spinal cord lesions, or clinical features indicating brainstem, cerebellum, or hemisphere involvement. The study protocol was approved by the Koc University Clinical Research Ethical Committee with the protocol ID of 2016.123.IRB2.077 and written informed consent was obtained from all participants.

CXCL5 Assessment

We asserted that CXCL5 levels were reduced in patient groups due to immunomodulating medications. Thus, secondly, we tested whether CXCL5 levels could be used for the determination of treatment responsiveness in MS. For this purpose, CXCL5 levels were measured in sera of 20 RRMS patients before and 3 months after fingolimod (0.5 mg/day) treatment. All patients experienced a 3-month washout period before fingolimod treatment. Serum CXCL5 levels were measured with an ELISA kit (Elabscience, Houston, TX, USA) according to the manufacturer's instructions, and results were expressed as pg/mL.

Statistical Analyses

Statistical analysis was performed using SPSS 20 software (SPSS Inc, Chicago, IL, USA) and the significance level was set at $p<0.05$. The variables were investigated using visual (histograms) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk's test) to determine whether they are normally distributed. Descriptive statistics were applied, and the characteristics of the groups were compared with paired or unpaired t-tests and ANOVA where appropriate.

RESULTS

CXCL5 levels were significantly higher in healthy controls compared to NMOSD ($p=0.04$) and MS-SCON ($p=0.01$) patients. However, CXCL5 levels of NMOSD

Table 1. Clinical and demographical data of participants

	RRMS (n=20)	NMOSDs (n=10)	MS-SCON (n=15)	Healthy control (n=14)
Gender (female/male)	14/6	8/2	11/4	11/3
Age, years (mean±SD)	34.35±8.29	32.20±8.55	37.20±8.26	32.78±8.95
Disease duration (mean±SD)	6.40±2.96	12.60±10.19	10.80±6.18	
EDSS (mean±SD)	1.9±1.28	3.6±2.19	2.46±1.76	

SD: Standard deviation; RRMS: Relapsing remitting multiple sclerosis; NMOSD: Neuromyelitis optica spectrum disorder; MS-SCON: MS presenting predominantly with spinal cord and optic nerve attacks.

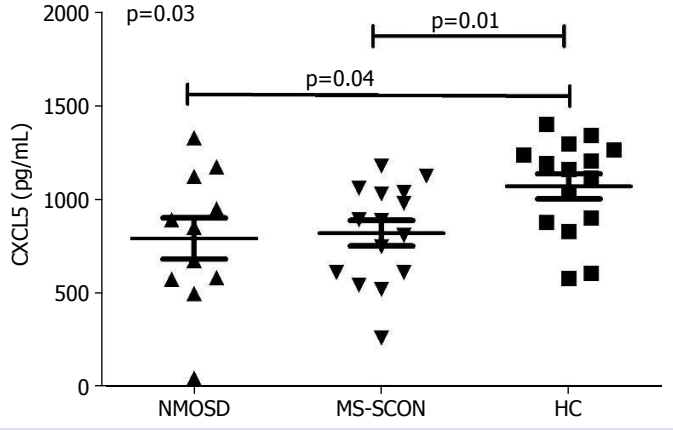

FIGURE 1. Serum CXCL5 levels of NMOSD, MS-SCON, and HC groups. CXCL5: C-X-C motif chemokine 5, NMOSD: Neuromyelitis optica spectrum disorder, MS-SCON: MS presenting predominantly with spinal cord and optic nerve attacks, HC: Healthy controls. Statistical comparison was done with ANOVA (p=0.03) and Tukey's *post-hoc* test (p=0.01, p=0.04). P<0.05 was considered for statistical significance. Bars indicate standard deviations and horizontal lines indicate mean values.

and MS-SCON patients were comparable (Fig. 1). Moreover, CXCL5 levels did not correlate with EDSS score, number of attacks, and disease duration, as assessed by Pearson's correlation test.

In keeping with our assertion, CXCL5 levels of RRMS patients in the washout period (921.37±343.33) were not significantly different from those of healthy controls (1073.77±257.14, p=0.24 with unpaired t-test) and a significant decrease was observed in CXCL5 levels after treatment (p=0.003 with paired t-test, Fig. 2). Post-treatment CXCL5 levels of RRMS patients (788.33±290.75) were significantly lower than those of controls (p=0.005, with unpaired t-test). There was no correlation between EDSS scores before and after treatment. Four patients who had more than one point increase in EDSS score and/or more than one attack during follow-up were considered treatment unresponsive. No significant difference was found between CXCL5 levels of treatment responsive and unresponsive patients before or after treatment (data not shown).

DISCUSSION

We may conclude that serum CXCL5 levels do not reflect the severity of NMOSD and MS and do not provide a prediction about prognosis after fingolimod

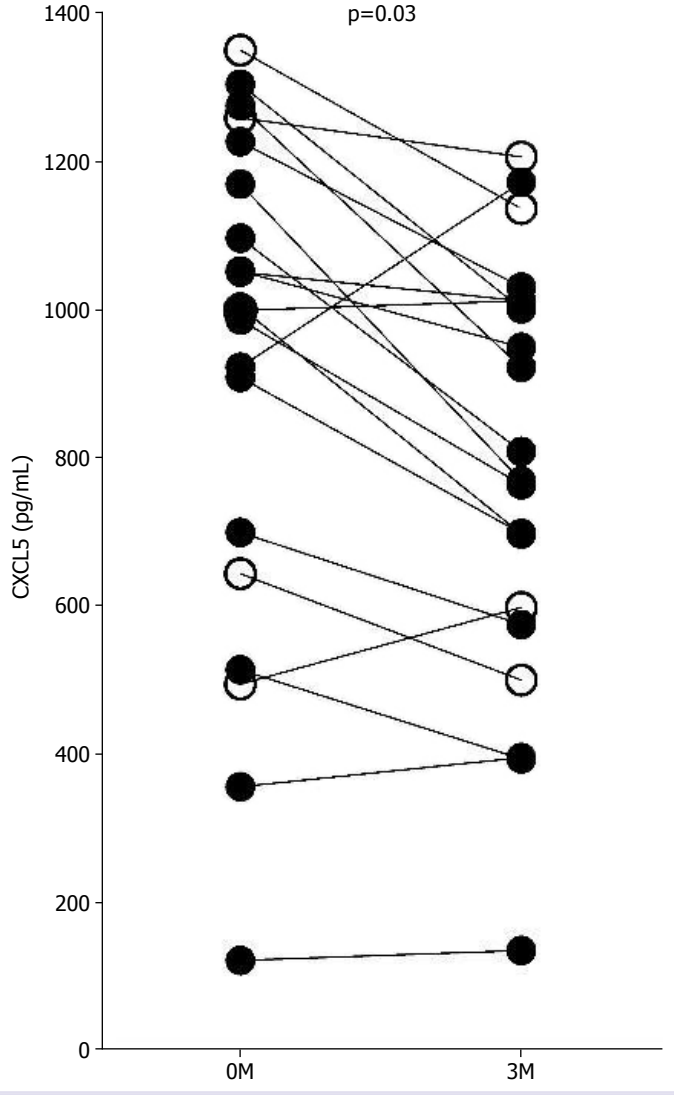

FIGURE 2. Serum CXCL5 levels of RRMS patients before (0M) and 3 months (3M) after fingolimod treatment. Statistical comparison was done with Student's t-test. P<0.05 was considered for statistical significance. Open circles represent drug unresponsive patient.

treatment. Our results also argue against the possible utility of CXCL5 in the differential diagnosis of MS presenting with clinical features reminiscent of NMOSD. It should be noted that immunomodulating drugs might plausibly have suppressed CXCL5 levels, thereby masking differences between patient groups and correlations between clinical features. Therefore, our results do not rule out the possibility that CXCL5 measurements in treatment-naïve patients might be utilized for diagnostic purposes. Furthermore, serum CXCL5 levels probably do not accurately reflect cerebral levels of CXCL5, which is more representative of

neuroinflammation in MS. Nonetheless, it has been shown that CSF CXCL5 levels did not show any differences between neuroinflammatory disease groups such as clinically isolated syndrome, RRMS, or secondary progressive MS patients [8].

Suppression of CXCL5 levels after fingolimod treatment supports the modulating role of this drug on innate immunity [9]. In chronic neuroinflammatory diseases, CXCL5 is released from both cerebral and blood-born innate immunity cells. Fingolimod has been shown to decrease CXCL5 expression in both astrocytes and microglia [10]. Moreover, MS patients under IVIg treatment also show reduced CXCL5 levels [11]. Our results also show that one of the actions of a wide array of immunomodulating treatment modalities (e.g. fingolimod, azathioprine, interferon-beta, glatiramer acetate, etc.) is suppression of CXCL5, thereby reducing recruitment of neutrophils into the brain parenchyma. This effect might be achieved through the inhibition of Th1 and Th17-type immune responses, which activate cells of innate immunity.

One of the limitations of our study is the low sample size, and the other is that NMO and MS-CON patients are under immunosuppressive therapy. However, NMO and MS-CON patients can generally be diagnosed after long-term follow-up makes it difficult to catch these patients during the untreated period. Although our study suggests that CXCL5 is not a good candidate biomarker for the differentiation of NMO and MS-CON, it provides a new clue about the mechanism of action of fingolimod treatment. Studies in this area with a larger cohort will shed light on fingolimod's effect on the innate immune system.

Conclusion

In conclusion, our results indicate that CXCL5 levels may possibly not be used as a prognostic and diagnostic biomarker in chronic MS or NMOSD patients under long-term treatment. However, whether serum/CSF measurements of CXCL5 levels in treatment-naïve patients may exhibit a biomarker quality still tends to be assessed.

Ethics Committee Approval: The Koc University Clinical Research Ethics Committee granted approval for this study (date: 30.05.2016, number: 2016.123.IRB2.077).

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

Authorship Contributions: Concept – ET, VY, RT; Design – VY; Supervision – ET, MK, RT; Fundings – ET, VY; Materials – RT, MK, HIA; Data collection and/or processing – ZK, HY, ES; Analysis and/or interpretation – ZK, VY; Literature review – ZK, VY; Writing – ZK, VY, ET; Critical review – MK, RT, ET.

REFERENCES

1. Kostic M, Dzopalic T, Zivanovic S, Zivkovic N, Cvetanovic A, Stojanovic I, et al. IL-17 and glutamate excitotoxicity in the pathogenesis of multiple sclerosis. Scand J Immunol 2014;79:181–6. [CrossRef]
2. Chang MS, McNinch J, Basu R, Simonet S. Cloning and characterization of the human neutrophil-activating peptide (ENA-78) gene. J Biol Chem 1994;269:25277–82. [CrossRef]
3. Rumble JM, Huber AK, Krishnamoorthy G, Srinivasan A, Giles DA, Zhang X, et al. Neutrophil-related factors as biomarkers in EAE and MS. J Exp Med 2015;212:23–35. [CrossRef]
4. Carlson T, Kroenke M, Rao P, Lane TE, Segal B. The Th17-ELR+ CXC chemokine pathway is essential for the development of central nervous system autoimmune disease. J Exp Med 2008;205:811–23.
5. Sheridan GK, Dev KK. S1P1 receptor subtype inhibits demyelination and regulates chemokine release in cerebellar slice cultures. Glia 2012;60:382–92. [CrossRef]
6. Yang T, Wang S, Zheng Q, Wang L, Li Q, Wei M, et al. Increased plasma levels of epithelial neutrophil-activating peptide 78/CXCL5 during the remission of Neuromyelitis optica. BMC Neurol 2016;16:96.
7. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, et al; International Panel for NMO Diagnosis. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology 2015;85:177–89. [CrossRef]
8. Lepennetier G, Hracsko Z, Unger M, Van Griensven M, Grummel V, Krumbholz M, Berthele A, et al. Cytokine and immune cell profiling in the cerebrospinal fluid of patients with neuro-inflammatory diseases. J Neuroinflammation 2019;16:219. [CrossRef]
9. Thomas K, Sehr T, Proschmann U, Rodriguez-Leal FA, Haase R, Ziemssen T. Fingolimod additionally acts as immunomodulator focused on the innate immune system beyond its prominent effects on lymphocyte recirculation. J Neuroinflammation 2017;14:41. [CrossRef]
10. O'Sullivan SA, O'Sullivan C, Healy LM, Dev KK, Sheridan GK. Sphingosine 1-phosphate receptors regulate TLR4-induced CXCL5 release from astrocytes and microglia. J Neurochem 2018;144:736–47.
11. Pigard N, Elovaara I, Kuusisto H, Paalavuo R, Dastidar P, Zimmermann K, et al. Therapeutic activities of intravenous immunoglobulins in multiple sclerosis involve modulation of chemokine expression. J Neuroimmunol 2009;209:114–20. [CrossRef]