BRIEF COMMUNICATION

# The CSF Levels of Neutrophil-Related Chemokines in Patients with Neuromyelitis Optica

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## Abstract

Pathologic findings showed that neutrophils played an important role in the pathogenesis of NMO. This study aims to investigate the CSF levels of neutrophil-related chemokines in NMO. CXCL1, CXCL5, and CXCL7 were measured in 95 patients with NMO, 15 patients with MS, 18 patients with GFAP astrocytopathy, and 16 controls. The CSF level of CXCL1, CXCL5, and CXCL7 was significantly elevated in the NMO group but not correlated with the patient clinical severity. Besides, the CSF CXCL1, CXCL5, and CXCL7 could act as biomarkers to distinguish NMO from MS with good reliability, especially the CXCL7.

## Introduction

Neuromyelitis Optica (NMO) and multiple sclerosis (MS) are chronic disabling idiopathic inflammatory demyelinating diseases (IIDDs).[1] Until Lennon *et al.* found the pathogenic anti-NMO antibody (aquaporin-4 antibody, AQP4) in 2004,[2] NMO had long been considered as a specific variation of MS. Since then, more and more evidences have supported that they were two distinctive diseases.[3] Pathological evidence has suggested that neutrophil infiltration in CNS lesions plays an important role in the pathogenesis of NMO,[4] while the inflammatory leukocyte infiltrated in MS lesions are mainly macrophages and T lymphocytes.[5,6] Chemokines play an important role in the migration of leukocytes to CNS.[7] CXCL chemokines (C-X-C motif chemokine ligand containing the glutamic acid, leucine, and arginine (ELR) motif) are the most important neutrophil-related chemokine families,[8] including CXCL1, CXCL5, CXCL7, CXCL10, and so on. The differences of these chemokines in NMO and MS patients may be an important factor which could account for the

pathological differences. A few studies have reported the cerebrospinal fluid (CSF) levels of these chemokines in patients with MS [9,10] and one study has reported the elevated plasma level of CXCL5 in NMO patients during remission,[11] but little is known about the CSF levels of these neutrophil-related chemokines in NMO patients during relapse. In the present study, we aimed to detect the levels of CXCL1, CXCL5, and CXCL7 in the CSF of patients with NMO and compared with patients with MS and autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy.

## Methods and materials

### Patients and controls

Ninety-five first diagnosis or relapsing NMO spectrum disorder (NMOSD) patients consistent with the 2015 Wingerchuk diagnostic criteria,[12] 15 relapsing–remitting MS patients consistent with the 2017 McDonald's diagnostic criteria,[13] 18 GFAP astrocytopathy patients based on the 2018 diagnostic criteria,[14] and 16 controls with non-inflammatory neurological diseases (including cervical or lumbar spondylopathy, peripheral vertigo and so on) from the Department of Neurology, Nanfang Hospital, Southern Medical University were included. All patients signed the informed consent.

### Preparation of CSF samples

All patients were subjected to lumbar puncture for CSF analysis within 3 days after admission. CSF samples were centrifuged within 30 minutes. The supernatant was then transferred to polypropylene tubes and stored at $-80°C$ until the assays were performed.

### Determination of CSF neutrophil-related chemokines

Enzyme linked immunosorbent assay (ELISA) kits were purchased and used to quantify the CSF concentration of CXCL1, CXCL5, and CXCL7(R&D Systems ELISA Kits), according to the manufacturers' instructions.

### Clinic evaluation

All NMOSD, MS, and GFAP patients were hospitalized during the first onset or relapses. The disease severity was assessed by Expanded Disability Status Scale (EDSS) scores. The clinical relapse of patients was defined as the onset of new symptoms that lasted at least 24 hours, and the EDSS score increased over 1.0 on admission.

### Statistical analysis

All statistical analyses were performed using SPSS version 20.0 (IBM Corp, Armonk, NY, USA). Data were expressed as mean (±standard deviation) or median (interquartile range). Kruskal–Wallis test was used for the comparison between groups. Correlations between EDSS and neutrophil-related chemokines were evaluated using Spearman's test. A value of $P < 0.05$ was considered statistically significant.

## Results

### Demographic and clinical features

The demographic data and clinical features of NMOSD patients (n = 95), MS patients (n = 15), GFAP patients (n = 18), and controls (n = 16) are shown in Table 1.

### CSF CXCL1, CXCL5, and CXCL7 Levels

CSF concentration of CXCL1, CXCL5, and CXCL 7 was detected in patients and controls groups and the data were shown in Table 1 and Figure 1. Whether CXCL1, or CXCL5, or CXCL7, the concentration of these three chemokines in the NMO group were significantly higher than in the other three groups. The CSF concentration of CXCL5 in the GFAP group was significantly higher than in the controls. Meanwhile, these three chemokines showed no significant difference between the MS group and the GFAP group.

### Receiver operating characteristic (ROC) curve analysis

We performed ROC analysis for CXCL1, CXCL5, and CXCL7 to distinguish NMOSD patients from MS patients in Figure 2. The area under curve (AUC) for CXCL1 was 0.740 (95% CI: 0.626-0.854, $P = 0.003$, best cut-off value was 12.55 pg/ml with sensitivity 70.5% and specificity 66.7%). The AUC for CXCL5 was 0.781 (95% CI: 0.629-0.934, $P < 0.001$, best cut-off value was 37.0 ng/ml with sensitivity 83.2% and specificity 73.3%). The AUC for CXCL7 was 0.819 (95% CI: 0.687-0.951, $P < 0.0001$, best cut-off value was 325.3 ng/ml with sensitivity 75.8% and specificity 86.7%).

### Correlations between EDSS and neutrophil-related chemokines

We analyzed the correlationship between EDSS, △EDSS (the difference of EDSS after and before relapse), and these three neutrophil-related chemokines in each patients

**Table 1.** The demographic data and clinical features of patients in each group

|  | NMO (n = 95) | MS (n = 15) | GFAP (n = 18) | CTLs (n = 16) |
| --- | --- | --- | --- | --- |
| Gender (male/female) | 13/82 | 9-Jun | 6-Dec | 11-May |
| Age | 43.9 ± 15.8 | 35.5 ± 11.6 | 41.7 ± 17.7 | 33.9 ± 2.9 |
| Clinical symptoms |  |  |  |  |
| Fever | 7 (7%) | 0 (0%) | 14 (78%) | — |
| Dizziness | 15 (16%) | 2 (13%) | 12 (67%) | — |
| Disorders of behavior or cognition | 1 (1%) | 2 (13%) | 10 (56%) | — |
| Eye pain or Vision loss | 30 (32%) | 4 (26%) | 1 (1%) | — |
| Abnormal feeling | 53 (56%) | 6 (40%) | 2 (11%) | — |
| Autonomic disturbances | 43 (45%) | 1 (7%) | 4 (22%) | — |
| Abnormal movements | 1 (1%) | 9 (60%) | 12 (67%) | — |
| Epilepsy | 6 (6%) | 1 (7%) | 2 (11%) | — |
| Vomit | 17 (18%) | 1 (7%) | 4 (22%) | — |
| Lesion location |  |  |  |  |
| Brain | 6 (6%) | 15 (100%) | 14 (78%) | — |
| Spinal cord | 74 (78%) | 4 (27%) | 4 (22%) | — |
| Brian and spinal cord | 7 (7%) | 4 (27%) | 2 (11%) | — |
| Course (day, interquartile range) | 12 (7, 30) | 2 (3, 4.5) | 10 (7, 15) |  |
| EDSS (median, interquartile range) | 3.0 (2.0, 6.5) | 2.0 (2.0, 2.5) | 3.5 (2.5, 4.5) | — |
| △EDSS (median, interquartile range) | 2.0 (1.0, 3.5) | 0.5 (0.5, 1.5) | 3.0 (2.0, 4.0) |  |
| CSF WBC (×10$^6$/L, median (minimum–maximum)) | 0 (0-420) | 24 (0-80) | 24 (0-110) | 0 (0-3) |
| CSF CXCL1 (pg/ml, mean ± SD) | 18.8 ± 11.1 | 11.9 ± 3.0 | 12.1 ± 3.7 | 8.9 ± 1.5 |
| CSF CXCL5 (ng/ml, mean ± SD) | 41.6 ± 6.5 | 31.1 ± 13.9 | 34.2 ± 11.6 | 17.7 ± 5.3 |
| CSF CXCL7 (ng/ml, mean ± SD) | 466.7 ± 237.8 | 284.3 ± 155.2 | 347.6 ± 257.3 | 226.0 ± 33.1 |

CSF, Cerebrospinal Fluid; NMO, Neuromyelitis optica; MS, Multiple sclerosis; GFAP, glial fibrillary acidic protein; CTLs, controls; WBC, white blood cell; EDSS, Expanded Disability Status Scale; △EDSS, the change of EDSS after relapse.

group, which is shown in Figure 3. CXCL1 had a significant negative correlation with EDSS ($r = -0.214$, $P = 0.038$) and △EDSS ($r = -0.246$, $P = 0.010$) only in NMO group. CXCL5 had a significant negative correlation with △EDSS ($r = -0.296$, $P = 0.004$) only in NMO group. CXCL7 had a significant positive correlation with EDSS ($r = 0.475$, $P = 0.047$) only in GFAP group.

## Discussion

In the present study, we measured the levels of CXCL1, CXCL5, and CXCL7 in the CSF of patients with NMOSD and compared with the patients with MS and GFAP astrocytopathy. All these three neutrophil-related chemokines were significantly higher in the NMO. This result is consistent with the pathological findings that the CNS lesions of NMO were mainly infiltrated by neutrophils.[4,15]

During the past two decades, more and more evidences had verified that NMO was a distinct disease from MS,[3] especially in the pathological findings.[6,16] Autopsy had revealed that NMO patients had much more severe neutrophil infiltration in the CNS lesions than MS patients, and neutrophil was a very important factor for the inflammatory damage to CNS.[17] It could account for why the clinical symptoms are more severe in NMO patients than in MS patients. It also suggested that neutrophils might play an important role in the pathogenesis of NMO. In recent years, more evidence showed that neutrophils displayed important functions for both innate and adaptive immune responses. Neutrophils can produce many cytokines and chemokines interacting with endothelial cells, dendritic cells, macrophages, NK cells, T cells, and B cells. We found CD40L and BAFF/APRIL systems were important for aggressive B cells and T-cell responses, and might stimulate B-cell activation in NMO.[18,19] Neutrophils could release the CD40L-related molecules BAFF and APRIL which facilitated the survival of plasma cells emerging from antibody responses in NMO.[20] Neutrophils activated the alternative complement pathway and released C5 fragments, which also further amplified neutrophil proinflammatory responses.[21] An *in vitro* study showed that C5a-preactivated neutrophils were critical for autoimmune-induced astrocyte dysregulation in NMOSD.[22] In an animal model of NMO, inhibiting neutrophil protease could reduce IgG induced brain damage.[23] But in the present study, the CSF neutrophils count was not shown in the result, as the CSF WBC in most of NMO patients were normal (less than $8 \times 10^6$/L) and it could not count how many neutrophils or lymphocytes were there.
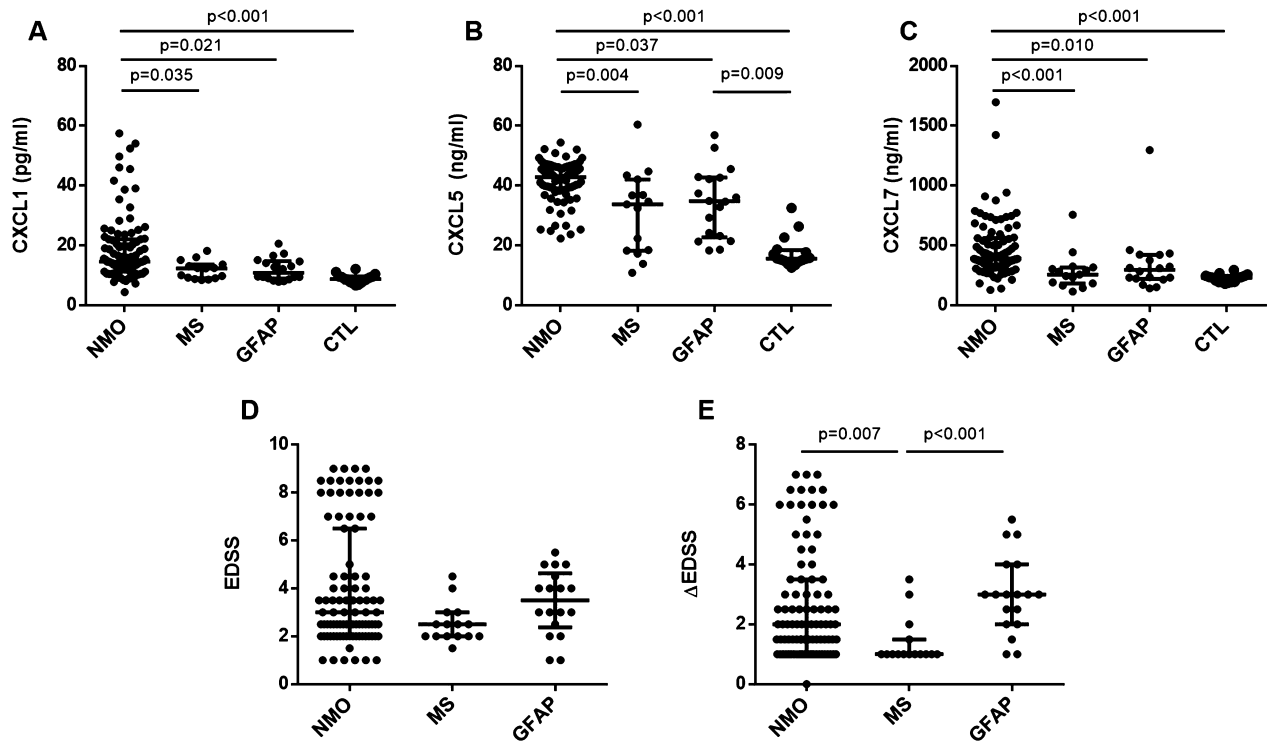
**Figure 1.** The CSF levels of CXCL1, CXCL5, and CXCL7 in NMOSD, MS, GFAP astrocytopathy patients and controls (A-C). The EDSS and △EDSS scores in these three patients groups (D, E).
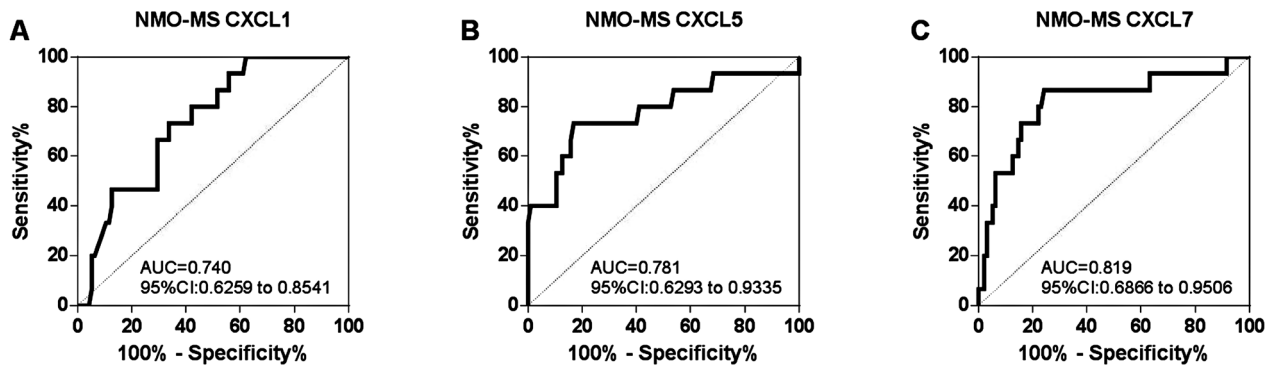


**Figure 2.** The ROC curve analysis of CSF CXCL1, CXCL5, and CXCL7 in NMOSD patients to distinguish from MS patients.

How does neutrophils migrate to CNS from peripheral blood? Neutrophil-related chemokines must play an important role.[7] CXCL1, CXCL5, and CXCL7 are the members belong to the CXC chemokines family, which mainly help the migration of neutrophils through their common receptor CXCR2.[8] Recently, studies on CXCL1, CXCL5, and CXCL7 mainly focus on the biomarker and pathogenesis of various malignant tumors,[24,25] but a few about CNS IIDDs existed. Rumble *et al.* reported elevated plasma CXCL5 in relapsing MS patients during

acute lesion formation and the expression of CXCL1 and CXCL5 correlated with measures of MS lesion burden and clinical disability.[9] Franciotta *et al.* reported that the CSF levels of CXCL1 and CXCL7 were similar as health controls in 14 MS patients.[10] Yang et al. reported elevated plasma CXCL5 in NMO patients during remission.[11] Little was known about these neutrophil-related chemokines in the CSF of relapse NMO patients. As both NMO and MS are central nervous IIDDs, we thought that the neutrophil-related
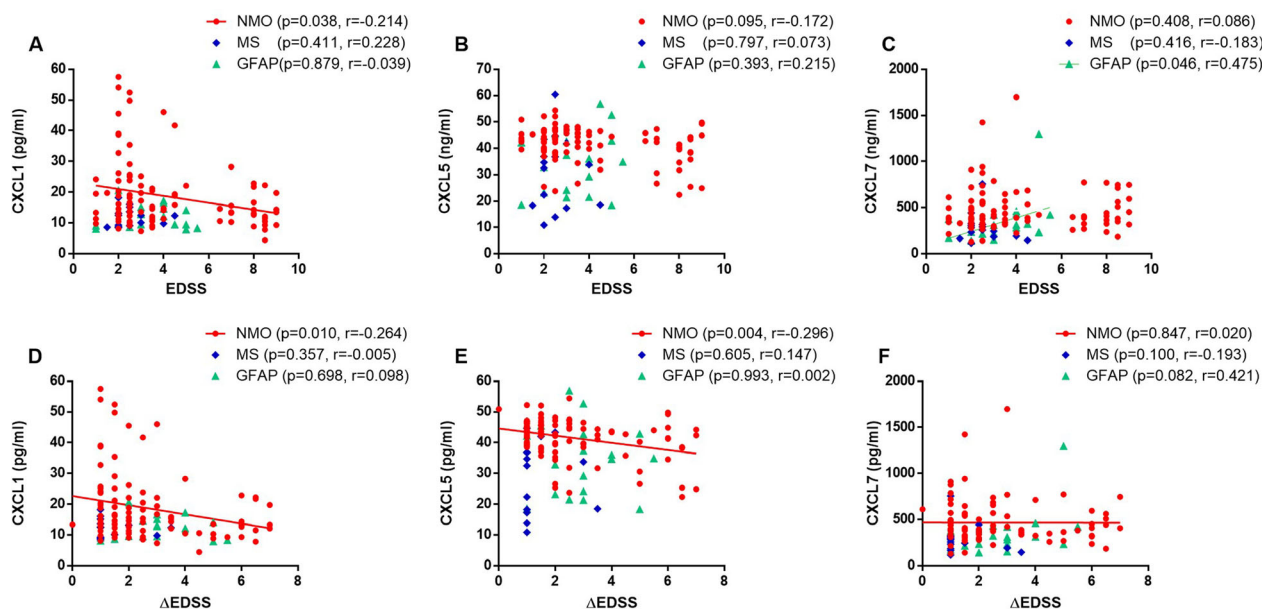
**Figure 3.** Correlation of CSF levels of CXCL1, CXCL5, and CXCL7 with the EDSS (A-C) and △EDSS (D-F) scores respectively in NMO, MS, and GFAP groups during relapse (Spearman's test).

chemokines released from central nervous system were more important for the central demyelinating lesions, and it might be reflected in the concentration of these chemokines in CSF.

As NMO and MS usually are very similar in clinic presentations, NMO used to be regarded as a variant of MS until the AQP4 antibody was discovered. But it was still sometimes difficult to distinguish these two diseases especially for the AQP4 antibody negative NMOSD patients.[26] So, we hoped to find some biomarkers that can identify NMO and MS. In the present study, these CSF neutrophil-related chemokines could act as diagnostic biomarkers for distinguishing NMO from MS, especially the CXCL7 had a good AUC value. In our previous studies, we had also detected some other valuable diagnostic biomarkers for NMO, such as neurofilament,[27] soluble syndecan-1 (sCD138),[28] soluble cluster of differentiation 27 (sCD27),[29] Chitinase-3-like-1 (alias YKL-40, yet not published), pyrin domain containing 3 (NLRP3) inflammasomes.[30] These biomarkers represented neuron injury, endothelial injury, B lymphocyte activation, microglia activation, and general inflammation respectively, and indicated that the pathogenesis of NMO was multifactorial. So, it could account for why the EDSS or △EDSS do not have positive correlations with these neutrophil-related chemokines.

GFAP astrocytopathy is a newly named disease in recent years, which is characterized by CSF positive anti-GFAP antibody.[14] However, it is still controversial whether GFAP antibody is the pathogenic antibody of this disease or secondary to the damage to astrocytes.[31,32] As some new evidences of pathology, NMOSD is also considered as an astrocytopathy.[33] In the present study, the CSF levels of CXCL1, CXCL5, and CXCL7 in the GFAP group were significantly lower than those in the NMO group, suggested that the pathological mechanisms of the two diseases were different.

There were some limitations in the present study. Firstly, compared with the cases of NMO group, the sample size of MS group, GFAP group, and control group was a little small. It is due to the higher incidence of NMO than MS in Asian populations.[34] Secondly, this is only a preliminary time/cross-sectional designed study. Only 13 of 95 NMOSD patients received lumbar puncture and re-evaluation of these neutrophil-related chemokines during follow-up. So it was difficult to evaluate the relationship between these chemokine changes and clinical recovery (data not shown). Finally, the exact effects of neutrophils in the pathogenesis of NMOSD are not clear and remains to be clarified.

## Conclusion

This study showed markedly higher levels of CSF CXCL1, CXCL5, and CXCL7 in the NMO. It could account for why NMO patients had much more severe neutrophil infiltration. It also suggested that neutrophils might play an important role in the pathogenesis of NMO. The CSF CXCL1, CXCL5, and CXCL7 could act as biomarkers to

distinguish NMO from MS with good reliability, especially the CXCL7.

## Acknowledgments

## Author Contributions

H. W., D. Z., and Y. P. conceived this study and designed the experiments. Z. L., J. C., Z. W., Y. W., and Y. P. collected the samples and clinical data. Z. L. and J. C. performed the experiments and analyzed the data. Y. P. wrote the manuscript and it was finally revised by D. Z. and H. W. All authors read and approved the final manuscript and agreed to submit it for publication.

## Conflict of Interest

All authors read and approved the final manuscript. They declare no conflicts of interest.

## Ethics Statement

The study protocol was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University. Each participant provided written informed consent.

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