Cerebrospinal Fluid Microglial Activation Markers in Patients With Neuromyelitis Optica

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Short paper

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Abstract

**Aim:** Microglia, the resident immune cells in the brain can be activated by inflammation in the central nervous system (CNS), then become significant sources of inflammatory mediators. However, little is known about whether microglia activation in neuromyelitis optica (NMO). The aims of this pilot study were to investigate the levels of the biomarkers of microglia activation, YKL-40, sCD163 and sCD14 in patients with NMO and the possible associations between those and Expanded Disability Status Scale (EDSS) scores.

**Methods:** We found significantly higher CSF YKL-40, sCD163 and sCD14 levels in NMO patients. The level of neurological disability was assessed by the EDSS scores.

**Results:** The levels of microglia biomarkers in NMO patients were positively correlated with Expanded Disability Status Scale (EDSS) scores, but not in MS and GFAP groups. Finally, we showed levels of CSF YKL-40, sCD163 and sCD14 were higher in NMO patients and positive relationship with disease severity of NMO.

**Conclusion:** These findings suggesting that microglial activation, may be implicated in the pathogenesis of NMO.

Introduction

Neuromyelitis optica (NMO), one of chronic disabling idiopathic inflammatory demyelinating diseases (IIDDs) of the central nervous system (CNS), was considered an optic-spinal form of multiple sclerosis (MS). Until 2004, specific antibodies (NMO-IgG) were suggested to be a biomarker of NMO, distinguishing it from conventional MS [1, 2]. The pathogenesis of NMO is not completely clarified; it is believed that the antibodies produced after the activation of B lymphocytes and the involvement of T cells and the participate of a large number of complements play a vital role. Recently, microglia active has been described in NMO [3, 4].

As an important resident neuroglia, microglia mediate a variety of immune responses in the CNS with interaction of peripheral immune cells [5]. It is generally believed that microglia originate from monocytes in the blood circulation [6]. Microglia, as the most abundant cells in the human CNS, once activated, can become an important source of inflammatory mediators, which can lead to obvious neuronal damage. Generally, activated microglia can lead to M1/M2 polarization. In the early stage, microglia differentiate into M1 phenotype and release pro-inflammatory cytokines, leading to inflammatory damage. In the later stage, it transformed into M2 phenotype, participates in the tissue repair process of inflammation [8, 9]. In recent animal experiments, it has been found that chemokines expressed by activated microglia can direct leukocytes to the axons of the CNS and cause damage [10]. Accordingly, it seems that neuroimmunity associated with microglia is highly associated with neuroinflammatory diseases.

Chitinase-3-like 1 (CHI3L1), alias YKL-40, mostly expressed by microglia, especially in the process of acute and chronic inflammation [11-13]. Increased levels of YKL-40 in CSF are associated with a variety of neuroimmune and neuroinflammatory diseases, such as MS, autoimmune encephalitis and stroke [14, 15]. CD14, a leukocyte differentiation antigen of monocytes, macrophages and activated neutrophils, is the receptor of Lipopolysacharide, including cell membrane type (mCD14) and serotype (sCD14) [8]. sCD14 is a pattern recognition protein, involving in cell death and clearance process [9]. In the CNS, CD163 is expressed in activation microglia, perivascular cells and meningeal macrophages. In the state of inflammation, membrane-bound CD163 falls off from the cell surface and became soluble CD163. Recent findings shown that sCD163 can play a role in downstream anti-inflammatory and antioxidant responses by inhibiting the proliferation and activation of T lymphocytes [16]. As the biomarkers of microglia activation, elevated levels of CSF or blood YKL-40, sCD14 and sCD163 were observed in patients with MS, certain CNS infections as well as brain aging and injury [16-24]. However, little is known about the microglia activation in NMO. In this present study, we
aimed to determine the levels of YKL-40, sCD163 and sCD14 in CSF in patients with NMO and to evaluate the association between these markers and the extended Disability status scale ((EDSS)) score.

Materials And Methods

2.1 Patients and Controls

106 first diagnosis or relapsing NMO patients consistent with the 2015 Wingerchuk diagnostic criteria[25], 15 relapsing–remitting MS patients fulfilling the 2017 McDonald’s diagnostic criteria[26], 20 Autoimmune Glial Fibrillary Acidic Protein astrocytopathy patients based on the 2018 diagnostic criteria[27] and 16 controls with non-inflammatory neurological diseases from the Department of Neurology, Nanfang Hospital, Southern Medical University were included. The clinical relapse of patients with MS and NMO was defined as the onset of new symptoms that lasted at least 24 hours, and the EDSS score increased over 1.0 on admission. All the patients enrolled in the study needed to be diagnosed by two neurologists. All samples were collected before treatment. The clinical characteristics and demographic of the patients are shown in Table 1.

2.2 Determination of CSF YKL-40, sCD14 and sCD163

After the patients were admission, all patients underwent lumbar puncture for CSF analysis before treatment. CSF samples were immediately centrifuged after collection in order to separated cells and larger particles, and then stored at −80°C until the assays were performed. Commercial Sandwich Enzyme linked immunosorbent assay (ELISA) kits were used to detect the CSF concentrations of YKL-40 (Quantikine ELISA, R&D Systems), sCD14 and sCD163 (Bender Med Systems GmbH, Vienna, Austria). ELISA assays were performed in accordance with the manufacturers’ instructions.

2.3 EDSS scores

EDSS scores were performed by two researchers on all enrolled patients at the initial stage of admission.

2.4 Statistical Analysis

SPSS 20.0 (IBM Corp, Armonk, NY, USA) was used for statistical analysis, and the data were expressed as mean ± standard deviation. Kruskal-Wallis test was used to analyze the differences of YKL-40, sCD14 and sCD163 levels among different subgroups. Pearson's test or Spearman's test were used to evaluate the correlation between YKL-40, sCD14, sCD163 levels and EDSS score. P < 0.05 was considered statistically significant.

Results

The demographic and clinical characteristics of the patients

As shown in Table 1, there were no significant difference in age and gender among the groups. The EDSS score and CSF white blood cell in the patients with NMO, MS and GFAP were no significant difference.

CSF YKL-40, sCD14 and sCD163 Levels

As the results shown in the Figure 1, the concentration of YKL-40, sCD14 and sCD163 were detected in the CSF from NMO (n=103) patients, MS (n=15) patients, GFAP (n=20) patients and controls (n=16). Mean YKL-40 (pg/ml) was 210.52±161.62 for NMO, 93.56±40.91 for MS, 129.11±46.03 for GFAP and 63.18±9.22 for control group. YKL-40 concentration in NMO group was significantly higher than that in MS group (p<0.005) and control group (p=0.008, Fig. 1A), but there is no significant difference between NMO and GFAP. The contents of YKL-40 in MS exceeded than in control group (p<0.05). Levels of sCD14 and sCD163 in NMO group were significantly higher than control group (p=0.03,
Besides, the obvious difference exists between NMO and GFAP, MS for the levels of sCD163 ($p=0.02$, $p<0.001$, Fig. 1B-C), that also being between CTLs and GFAP, MS ($p=0.001, p<0.001$). When comparing the levels of sCD14 among subgroups of inflammatory demyelinating diseases (IDDs), the levels of sCD14 were not significant difference within groups, as well as compared with the controls group.

**Correlations between YKL-40, sCD14 and sCD163**

Considering that the concentration of YKL-40, sCD14 and sCD163 were rising in NMO, GFAP and MS group, we examined the correlation between them. We found that the YKL-40 was significantly correlated with sCD14 ($r=0.89, p<0.0001$) and sCD163 ($r=0.77, p<0.0001$) in NMO group, another positive result was also obtained between sCD14 and sCD163 in NMO ($r=0.77, p<0.0001$). However, there were no any correlations between YKL-40, sCD14 and sCD163 in GFAP and MS group.

**Correlations between CSF profiles and EDSS Score**

As shown Figure 2A, the severity of the disease in patients with NMO toward an increased with elevated levels of YKL-40, sCD14 and sCD163 ($p<0.05$). However, CSF YKL-40, sCD14 and sCD163 levels were not associated with EDSS scores in MS and GFAP patients (Fig. 2B, C).

**Discussion**

In this case–control study, we confirmed that the concentrations of YKL-40, sCD14 and sCD163 were increased in NMO patients. Moreover, these biomarkers were all significantly associated with the severity of the disease. To our knowledge, this study reported for the first time about the changes of YKL-40, sCD14 and sCD163 levels in CSF of patients with NMO.

NMO is an autoimmune disease characterized by optic neuritis and myelitis. The exact mechanism of NMO neuronal damage is still unclear. The key factors that dominate the current speculation are microglia or macrophage activation, astrocyte damage caused by AQP4 antibodies, secondary demyelination of nerve axons, and lactic acid accumulation in damaged mitochondria. Microglia, also known as CNS macrophages, are the main immune surveillance cells in the CNS and play a key role in the process of inflammation. The most prominent feature of microglia is the rapid activation when under stress conditions such as injury, inflammation, neurodegeneration, infection and brain tumor. Microglial activation, as revealed in CSF by sCD14, sCD163 and YKL-40 have well known implications in brain diseases, such as MS, intracranial infection and neurodegenerative diseases.[7, 28].

Interleukin-6 (IL-6) and type I interferon family members, including interferon-α (IFN-α) and interferon-β (IFN-β), are classic inflammatory promoters and have been shown to be key inflammatory mediators in the pathogenesis of central nervous system inflammatory demyelinating diseases such as NMO and MS. It has also been confirmed that IL-6 is an important cytokine causing the recurrence of NMOSD.[29] IL-6 and IFN-α can lead to the activation of microglia and participate in the regulation of microglia related inflammation. Up to now, more and more evidence shows that highly reactive microglia is a key feature of NMO.[30-33].

Recent findings query the notion that microglial is functional only when the CNS in a diseased state. The current understanding is microglia not only have a simple reacting to the injury, infection or pathological state, evidence shows that microglia can repair the structure of CNS, refine regulate the connectivity of neural circuits and networks, but also play a role in the formation of nervous system plasticity.[34] However, whether the effect of microglia activation is beneficial or harmful remains to be further clarified.

Conspicuous activation of microglia has been observed in the actively demyelinating spinal cord lesions of patients with NMOSD, considered to be one of the pathogenic mechanisms, and it is also proposed that limit the adverse effects of
microglia activation as a future treatment strategy worthy for further investigation [35, 36]. And the same performance was found in animal experiments. Injection of serum IgG from NMO patients into the spinal cord or optic nerve of the experimental rats, can lead to NMO-like pathological changes, including significant activation of microglia around these lesion[37-39]. In another animal experiments, they observed that exogenous injection of patients’ serum AQP4-IgG could induce marked microglial activation, and further co-immunostaining revealed that these activated microglia were in close to astrocytes in the spinal cord lesions, suggesting that microglia can phagocytose AQP4 positive astrocytes[40]. Through the observation of these human or animal models, we found that microglia activation can directly affect the function and survival of astrocytes, as well as chemotactic highly inflammatory factors, aggravate the inflammatory response of the CNS, thus promoting the occurrence of NMOSD.

In the present study, the levels of these microglia activation markers in CSF of patients with NMO were positively correlated with EDSS scores, further clarifying that microglia activity were coincided with the severity of disease. Suggesting that YKL-40, sCD14 and sCD163 can be used to assess the severity of NMO and serve as a marker to monitor the effect of treatment and predict the prognosis of the disease.

**Conclusions**

In this study, we first reported the increased levels of sCD14, sCD163 and YKL-40 in CSF of patients with NMO, and the levels of these markers were positively correlated with EDSS score, suggesting that microglia activation is involved in the pathogenesis of NMO. In order to fully understand the role of microglia activation in NMO, more mechanistic and experimental studies are needed in the future.

**Declarations**

**ETHICS STATEMENT**

This study and the use of human material for study were approved by the Ethics Committee of Nanfang Hospital of Southern Medical University. After a detailed explanation of the purpose and procedure of the study, written informed consent was obtained for each participant.

**CONSENT FOR PUBLICATION**

This study is original and has not been published elsewhere.

**CONFLICT OF INTEREST**

The authors declare no conflict of interests.

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Tables

Table 1. Demographic and clinical features of the patients and controls.
|                          | NMO (n=103) | MS (n=15) | GFAP (n=20) | CTLs (n=16) |
|--------------------------|-------------|-----------|-------------|-------------|
| Gender (Male/Female)     | 13/90       | 6/9       | 13/7        | 11/5        |
| Age (years)              | 43.99±15.76 | 35.53±11.56 | 38.70±11.50 | 33.90±2.00 |
| Clinical symptoms        |             |           |             |             |
| Fever                    | 8 (8%)      | 0 (0%)    | 16 (80%)    | –           |
| Dizziness                | 17 (17%)    | 2 (13%)   | 13 (65%)    | –           |
| Disorders of behavior or cognition | 1 (1%)   | 2 (13%)   | 12 (60%)    | –           |
| Eye pain or Vision loss  | 33 (32%)    | 4 (26%)   | 1 (0%)      | –           |
| Abnormal feeling         | 56 (54%)    | 6 (40%)   | 2 (10%)     | –           |
| Autonomic disturbances   | 45 (44%)    | 1 (7%)    | 5 (25%)     | –           |
| Abnormal movements       | 1 (1%)      | 9 (60%)   | 13 (65%)    | –           |
| Epilepsy                 | 7 (7%)      | 1 (7%)    | 2 (10%)     | –           |
| Vomit                    | 18 (17%)    | 1 (7%)    | 5 (25%)     | –           |
| Lesion location          |             |           |             |             |
| Brain                    | 8 (8%)      |           | 15 (100%)   | 16 (80%)    | – |
| Spinal cord              | 81 (79%)    |           | 4 (26%)     | 4 (20%)     | – |
| Brain and spinal cord    | 9 (8%)      |           | 4 (26%)     | 2 (10%)     | – |
| CSF WBC×106 (median (minimum-maximum)) | 0 (0-420) | 24 (10-67) | 28.75 (8-96) | 0 (0-3) |
| CSF YKL-40 (pg/ml, mean±SD) | 210.52±161.62 | 93.56±40.91 | 129.11±46.03 | 63.18±9.22 |
| CSF sCD14 (pg/ml, mean±SD) | 87.23±56.85 | 71.66±26.20 | 85.16±38.24 | 58.14±7.66 |
| CSF sCD163 (pg/ml, mean±SD) | 68.22±24.11 | 79.04±9.70 | 75.97±16.54 | 55.76±9.48 |
| EDSS scores (median (minimum-maximum)) | 3,(0,9) | 2,(1,3) | 3, (1, 5.5) | – |

CSF: Cerebrospinal Fluid; NMO: Neuromyelitis optica; MS: Multiple sclerosis; CTLs: controls; WBC: white blood cell.

**Figures**
Figure 1

The levels of CSF YKL-40 sCD14 and sCD163 are shown. (A) CSF YKL-40 concentrations in NMO patients were significantly higher than in MS and controls (p < 0.05; p = 0.006), CSF YKL-40 concentrations in MS were also significantly higher than in controls (p < 0.05). (B) CSF sCD14 concentrations in NMO patients were significantly higher than in controls (p = 0.03). (C) CSF sCD14 concentrations in NMO patients were significantly higher than in GFAP, MS and controls (p=0.002; p< 0.001; p=0.006); CSF sCD14 concentrations in GFAP and MS patients were significantly higher than in controls (p<0.001; p<0.001).
Figure 2

Association between YKL-40 and cytokines in CSF and EDSS scores are shown. CSF levels of YKL-40 (A), sCD14 (B) and sCD163 (C) in NMO patients are associated with EDSS scores (p<0.005; p<0.005; p<0.05).
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Association between YKL-40 and cytokines in CSF and EDSS scores are shown. CSF levels of YKL-40 (A), sCD14 (B) and sCD163 (C) in NMO patients are associated with EDSS scores (p<0.005; p<0.005; p<0.05).