Elevated blood and cerebrospinal fluid biomarkers for microglial activation and blood-brain barrier disruption in anti-NMDA receptor encephalitis

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Short Report

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Abstract

Background

Anti-NMDA receptor encephalitis is an autoimmune disease characterized by complex neuropsychiatric syndrome and cerebrospinal fluid (CSF) NMDAR antibodies. Triggering receptor expressed on myeloid cells 2 (TREM2) has been reported to be associated with inflammation of the CNS. Matrix metalloproteinase-9 (MMP9) and the cluster of differentiation (CD44) were measured to evaluate the blood-brain barrier (BBB) permeability of anti-NMDAR encephalitis. The roles of the microglia activation and disruption of BBB in anti-NMDAR encephalitis are not well known.

Findings:

In this work, we detected the increased expression level of CSF sTREM2, CSF and serum CD44, and serum MMP9 in anti-NMDAR encephalitis patients, compared with control groups. CSF sTREM2 levels were positively related to both the CSF CD44 levels (r = 0.702, p < 0.0001) and serum MMP9 levels (r = 0.428, p = 0.021). In addition, CSF sTREM2 levels were related to the clinical parameters (mRS scale, r = 0.422, p = 0.023, and GCS scores, r=-0.401, p = 0.031).

Conclusion

The increased CSF sTREM2 levels and CD44, and MMP9 in serum or CSF showed evidence of the activated microglia and the disruption of BBB in anti-NMDAR encephalitis, expanding the understanding of the neuroinflammation in this disease. These factors mentioned above may be considered novel targets for intervention or novel potential diagnostic biomarkers.

Introduction

Anti-N-methyl-D-aspartate receptor (NMDA) encephalitis is an autoimmune-mediated disease characterized by a complex neuropsychiatric syndrome, including rapidly progressive psychiatric symptoms or cognitive impairment, seizures, abnormal movements, or coma of unknown cause (1–3). It is generally believed that antibodies against NMDAR are produced by tumors, viral infections, or other causes and bind inversely to anti-NMDA receptors on the surface of neurons in the central nervous system, leading to cross-linking and internalization of receptors leading to pathogenesis(1).

Current brain biopsy or autopsy research and clinical evidence showed that Anti-NMDAR encephalitis patients at the acute stage (around 3 months or longer) had infiltration of immune cells, while the neuroinflammation and the role of microglial activation still have not been explored (4). In addition, unlike other autoimmune-mediated demyelinating diseases, most patients at the recovery stage showed a large
resolvent of symptoms and a minimal presence of inflammation (both MRI changes or CSF pleocytosis) (5), which shows a lack of understanding in neuroinflammation of Anti-NMDAR encephalitis.

The triggering receptor expressed on myeloid cell 2 (TREM2) is an immune receptor that is expressed abundantly by microglia in the central nervous system (CNS) and is involved in key immune-related functions of microglia activation (6–8). The soluble TREM2 (sTREM2), released into the extracellular space (e.g., CSF and serum), is a soluble fragment of TREM2 shedding by ADAM proteases (9). Therefore, increased levels of sTREM2 represent an elevated inflammation reaction (10).

The cluster of differentiation (CD) 44 is a type 1 transmembrane receptor and its standard form is widely expressed in the majority of immune cells (11). As a multi-faceted receptor, CD44 exists in intracellular and soluble forms (12). By mediating the adhesion to the extracellular matrix hyaluronan, CD44 recruits the antigen-activated T lymphocytes, and inflammatory agents stimulated monocytes (13).

Matrix metalloprotease (MMP) 9, a Zn$^{2+}$ dependent endopeptidase that can cleave type IV collagen in the extracellular matrix of BBB, is induced by inflammatory cytokines leading to the disruption of the blood-brain barrier (BBB) (14, 15). Although the disruption of the BBB is present in a variety of encephalitis, the increased markers (CD44 and MMP9) which are reflected in the BBB disruption in anti-NMDAR encephalitis are first reported in our research.

In this study, we measured the concentrations of the above biomarkers in CSF and serum of anti-NMDAR encephalitis patients to explore the relationship between the microglia activation, disruption of BBB, and anti-NMDAR encephalitis, providing sights for potential diagnostic markers and pathogenesis.

**Methods**

**Study design, participants, and sample collection**

In this study, 29 anti-NMDAR encephalitis and 15 control patients from the Neuroinfection and Neuroimmunology Center, Department of Neurology, Beijing Tiantan Hospital were enrolled between Jan. 2017 and Sep. 2021. The anti-NMDAR encephalitis patients were diagnosed according to the revised anti-NMDAR encephalitis diagnosis criteria of 2016, based on the clinical manifestations and identification of CSF antibodies against the GluN1 subunit of NMDAR by cell-based analysis. The control patients (15 cases) with non-inflammatory neurological diseases (OND) were selected, including benign intracranial hypertension (n= 7), peripheral neuropathy (n= 2), diabetic retinopathy (n= 1), intracranial hypotension headache (n= 1), anxiety disorders (n= 3), hypertension (n= 1).

All the samples were collected before treatment when anti-NMDAR encephalitis patients were in an acute phase. The CSF and the serum samples were immediately centrifuged at 1000 g for 10 min and stored in the supernatant at -80 °C until detection by ELISA assays.
Ethics approval and consent to participate

Experiments were carried out following the ethical principles established in the Declaration of Helsinki. Patients (or their representatives) were informed about this study and gave written informed consent. The study was approved by the Ethics Committee of Beijing Tiantan Hospital, Capital Medical University (No. KY2015-031-02). All participants were written and obtained their informed consent before proceeding with the study.

Detection of biomarkers by ELISA

Enzyme-Linked Immunosorbent Assay (ELISA) kits were applied to measure the concentration of sTREM2 (Cat. ab224881, Abcam), CD44 (Cat. CSB-E11846h, Cusabio), and MMP9 (Cat. CSB-E08006h, Cusabio) in humoral fluids. All detections and measurements were performed following the manufacturer’s instructions and every standard and sample was assayed in duplicate.

Statistical Analysis

All statistical analyses were conducted using SPSS version 24.0 (IBM, Armonk, NY, US) and Prism (GraphPad software 8.0 version). Data are presented as mean±SD or the median with an interquartile range based on the normality test. The differences in serum CD44 levels between anti-NMADR encephalitis and control groups were assessed with an independent two-sample t-test. The Mann-Whitney test was applied when comparing the differences in the CSF CD44 levels, the CSF sTREM2, and the serum MMP9 levels between anti-NMADR encephalitis and control groups. Correlation coefficients between sTREM2, CD44, MMP9, mRS scale, GCS scores, and Qalb were calculated using Spearman’s two-tailed correlation test when one of the variables did not satisfy normal distribution. The p-value<0.05 was considered statistically significant.

Results

Patient characteristics

The clinical data and the concentration of the biomarkers from patients with anti-NMADR encephalitis (n=29, 15 male and 14 female) and OND control (n=15, 4 male and 11 female) are presented in Table 1. Diagnoses of anti-NMDAR encephalitis were confirmed by two doctors according to the revised diagnostic criteria (2016). All OND controls are negative for specific CSF antibodies. The mRS scale was used to measure the degree of disability or dependence in daily activities and the GCS score was applied to assess consciousness level. The Qalb was used to measure the degree of BBB permeability.
sTREM2, MMP9, and CD44 are increased in the extracellular fluid of anti-NMDAR encephalitis patients

The median levels of CSF sTREM2 are 49.15 (37.48-97.54) ng/mL in the anti-NMDAR encephalitis group, and 24.75(14.45-32.88) ng/mL in the OND control group. The median levels of serum sTREM2 are 25.64(17.50-42.92) ng/mL in the anti-NMDAR encephalitis group, and 24.55(13.32-34.73) ng/mL in the control group. The level of CSF sTREM2 was increased significantly in the anti-NMDAR encephalitis patients than that of the control group (Fig.1A, p<0.0001), but the serum sTREM2 levels did not differ in two groups (Fig.1B).

The median levels of serum MMP9 are 306.09 (209.68-527.40) ng/mL in anti-NMDAR encephalitis group, and 210.63 (179.56-268.60) ng/mL in control group. The serum MMP9 levels increased markedly in the anti-NMDAR encephalitis group, compared with the control group (Fig.1C, p=0.044).

In addition, the median levels of CSF CD44 are 42.55(31.33-69.47) ng/mL in anti-NMDAR encephalitis group, and 15.95(12.09-72.15) ng/mL in control group. The median levels of serum CD44 are 13.2(10.92-17.33) ng/mL in anti-NMDAR encephalitis group, and 7.72(4.26-12.17) ng/mL in control group. The CD44 levels in both CSF (Fig.1D, p=0.018) and serum (Fig1E, p<0.0001) elevated significantly were observed between the anti-NMADR encephalitis group and the control group.

sTREM2 levels of anti-NMDAR encephalitis patients correlated with inflammatory factors and clinical parameters.

The CSF sTREM2 is related to the anti-NMDAR encephalitis inflammatory factors. As shown in Fig.2, significant positive correlations were explored between the CSF sTREM2 expression level and the serum sTREM2 (r=0.426, p=0.021, Fig.2A), the CSF CD44 (r=0.702, p<0.0001, Fig.2B), and the serum MMP9 (r=0.428, p=0.021, Fig.2C) expression levels in the anti-NMDAR encephalitis patients. Besides, the CSF and serum sTREM2 expression levels are related to the clinical parameters. As for the CSF sTREM2 expression level, there were significantly positive correlation between the CSF sTREM2 expression level and mRS scale (r=0.422, p=0.023, Fig.2D) and negatively correlation between the CSF sTREM2 expression level and GCS scale (r=-0.401, p=0.031, Fig.2E) in the anti-NMDAR encephalitis patients. As for the serum sTREM2 expression level, the serum sTREM2 expression level was notable positively correlated to the Age in anti-NMDAR encephalitis patients (r=0.407, p=0.028).

Both CSF CD44 and serum MMP9 are correlated with clinical parameters.
Other inflammatory factors (CD44 and MMP9) are related to the clinical parameters, including mRS, GCS, and Qalb. As for the CSF CD44 expression level, a positive correlation was shown between the CSF CD44 expression level and the mRS scale ($r=0.509$, $p=0.005$, Fig.3A), and a negative correlation was found between the CSF CD44 expression level and GCS scale ($r=-0.382$, $p=0.041$, Fig.3B). There was also a positive correlation between the CSF CD44 expression level and the serum MMP9 expression level ($r=0.467$, $p=0.011$, Fig.3C). As for the serum MMP9 expression level, a positive correlation was demonstrated between the serum MMP9 expression level and the Qalb in anti-NMADR encephalitis patients ($r=0.493$, $p=0.007$, Fig.3D).

**Discussion**

This study is the first to show evidence of activated microglia in anti-NMDAR encephalitis, accompanied by BBB disruption, and is closely related to the clinical features. The results indicated that 1) the increased concentration of sTREM2, CD44, and MMP9 in CSF and serum of the anti-NMDAR encephalitis compared with the control groups; 2) the above biomarkers are related to clinical parameters (mRS scale, GCS score, and Qalb; 3) the activation of microglia and disruption of BBB take a role in the pathogenesis of anti-NMDAR encephalitis.

The soluble form of TREM2 (sTREM2) is reported to be increased in the cerebrospinal fluid of patients with Alzheimer’s disease, multiple sclerosis, and neurosyphilis, as a marker of microglial activation among neuroinflammation (16-21). Activation of microglia promotes the production of cytokines, chemokines, and matrix metalloproteinases(8), which disrupt the blood-brain barrier and lead to the entry of blood-derived immune cells, cytokines and supplements into the central nervous system, further activating microglia and increasing neuronal damage (22). Tobias Zrzavy et.al. confirmed a topographic distribution of inflammation in two untreated anti-NMDAR encephalitis patients, accompanied by infiltrated immune cells (CD3+/CD8+ T cells and CD79a+ B cells/plasma cells)(23). However, the brain MRI showed a lower frequency of abnormal MRI findings(5). In this study, we found the concentration of sTREM2 is increased in CSF of the anti-NMDAR encephalitis patients compared with controls, showing microglia activation may take a role in pathogenesis in the acute stage of anti-NMDAR encephalitis.

Previous studies showed that damaged BBB was related to a poor prognosis(24).

To further explore this hypothesis, we found that CD44 and MMP9 levels were also increased in CSF and serum, respectively. The CSF STREM2 level is positively correlated to both CSF CD44 levels and serum MMP9 levels. Under the inflammatory environment, CD44 is upregulated and secreted by M1 macrophages and modulates leukocyte adhesion, migration, and functional phenotype (13). In addition, CD44 is regulated with hyaluronan, a key biophysical component of BBB, impairing the barrier integrity of brain microvascular endothelial cells through a CD44-dependent pathway (25). Furthermore, studies have shown that MMP-9 could cause the leakage of the BBB (14). The level of serum MMP9 was related to Qalb, a common clinical indicator to evaluate the destruction of the BBB, in anti-NMADR encephalitis patients. Thus, the high levels of CSF CD44 and serum MMP9 in anti-NMDAR encephalitis patients...
showed evidence of (12) BBB disruption in anti-NMDAR encephalitis, accompanied the microglia activation.

mRS scales and GCS scores are used to estimate the severity of clinical characteristics in anti-NMDAR encephalitis patients. Both the level of CSF sTREM2 and CD44 are positively related to the mRS scales and negatively related to the GCS scores, which showed the potential for indicating the severity of the anti-NMDAR encephalitis.

Above all, our study first reported the increased level of CSF sTREM2, CSF CD44, and serum MMP9 in anti-NMDAR encephalitis and demonstrate the microglia activation within BBB disruption along the acute stage. The results showed a sight of microglia taking a role in pathogenesis and the damaged BBB involved in the pathology, which provides a new perspective for the potential target for the treatment and biomarkers of diagnosis.

**Abbreviations**

sTREM2, soluble triggering receptor expressed on myeloid cells 2; CSF, cerebrospinal fluid; MMP9, metalloproteinase-9; CD44, cluster of differentiation 44; BBB, blood-brain barrier; NMDA, N-methyl-D-aspartate receptor.

**Declarations**

**Acknowledge**

We thank Ms. Yuan Cai for her assistance in statistical analysis, Dr. Lin Zhao, Ms. Qiaoxi Dong, and Ms. Yunting Kou for their support. We thank Prof. Xianhao Xu (Beijing Hospital) inspired us to carry out this research.

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**Availability of data and materials**

The raw data supporting the conclusions of this article are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

Experiments were carried out following the ethical principles established in the Declaration of Helsinki. Patients (or their representatives) were informed about this study and gave written informed consent. The study was approved by the Ethics Committee of Beijing Tiantan Hospital, Capital Medical University (No.
KY2015-031-02). All participants were written and obtained their informed consent before proceeding with the study.

Author's contributions

HXC and XHZ contributed to the concept development and study design. JM, JLS, and TSG prepared reagents, performed experiments, and analyzed data. KF, NF, XXW, and HBW selected and clinically characterized patients. YZW and YX carried out statistical analyses. LLY obtained financial support. HXC and JM coordinated the study and draft the manuscript. All authors read and approved the final manuscript.

Consent for publication

Not Applicable.

Competing interests

All the authors declare no potential conflict of interest concerning the research, authorship, and/or publication of this article.

References


Tables
Table 1. Clinical and demographic characteristics of participants.

<table>
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<tr>
<th>Subject details</th>
<th>Con (n=15)</th>
<th>anti-NMDAR encephalitis (n=29)</th>
</tr>
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<tr>
<td>Age, mean ± SD, y</td>
<td>43.13±9.91</td>
<td>33.83±13.87</td>
</tr>
<tr>
<td>Sex, No. (%)</td>
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<td></td>
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<tr>
<td>Male</td>
<td>4(26.7%)</td>
<td>15(51.72%)</td>
</tr>
<tr>
<td>Female</td>
<td>11(36.4%)</td>
<td>14(48.28%)</td>
</tr>
<tr>
<td>adapted modified Rankin Scale (mRS) score</td>
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<td></td>
</tr>
<tr>
<td>Score 0, no symptoms</td>
<td>–</td>
<td>6(20.69%)</td>
</tr>
<tr>
<td>Score 1, nondisabling symptoms</td>
<td>–</td>
<td>10(34.49%)</td>
</tr>
<tr>
<td>Score 2, minor symptoms</td>
<td>–</td>
<td>1(3.45%)</td>
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<tr>
<td>Score 3, moderate symptoms</td>
<td>–</td>
<td>3(10.34%)</td>
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<tr>
<td>Score 4, moderately severe symptoms</td>
<td>–</td>
<td>3(10.34%)</td>
</tr>
<tr>
<td>Score 5, severely disabled</td>
<td>–</td>
<td>6(20.69%)</td>
</tr>
<tr>
<td>Score 6, dead</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Glasgow Coma Scale (GCS)</td>
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<tr>
<td>GCS 13-15, Mild head injury</td>
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<td>19(65.50%)</td>
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<tr>
<td>GCS 9-12, Moderate head injury</td>
<td>–</td>
<td>5(17.25%)</td>
</tr>
<tr>
<td>GCS 3-8, Severe head injury</td>
<td>–</td>
<td>5(17.25%)</td>
</tr>
<tr>
<td>CSF sTREM2 (ng/mL), median(IQR)²</td>
<td>24.75(14.45-32.88)</td>
<td>49.15 (37.48-97.54)***</td>
</tr>
<tr>
<td>Serum sTREM2 (ng/mL), median(IQR)²</td>
<td>24.55(13.32-34.73)</td>
<td>25.64(17.50-42.92)ns</td>
</tr>
<tr>
<td>CSF CD44 (ng/mL), median(IQR)²</td>
<td>15.95(12.09-72.15)</td>
<td>42.55(31.33-69.47)*</td>
</tr>
<tr>
<td>Serum CD44 (ng/mL), median (IQR)b</td>
<td>7.72(4.26-12.17)</td>
<td>13.2(10.92-17.33)***</td>
</tr>
<tr>
<td>Serum MMP9 (ng/mL), median(IQR)²</td>
<td>210.63(179.56-268.60)</td>
<td>306.09(209.68-527.40)*</td>
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<tr>
<td>Qalb (mean ± SD, damage %)</td>
<td>3.89±2.66</td>
<td>5.64±2.92</td>
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</table>

Data are expressed as mean±SD or median±SD (IQR) for continuous variables.

²Assessed by the Mann-Whitney test. bAssessed by the independent two-sample t-test. *p<0.05, ***p<0.001, ns, .
y, year; CSF, cerebrospinal fluid; sTREM2, soluble Triggering receptor expressed on myeloid cell-2; CD44, Cluster of differentiation 44; MMP9, matrix metalloproteinase 9; Qalb, the cerebrospinal fluid (CSF)/serum quotient of albumin.

Figures

Figure 1

Levels of sTREM2, MMP9, and CD44 in serum and CSF between anti-NMDAR encephalitis and patients.

(A-B). The CSF sTREM2 levels were significantly increased (p<0.0001) in the anti-NMDAR encephalitis group, while the serum sTREM2 levels showed no differences, compared with the controls. (C) The serum MMP9 levels were higher (p=0.044) in the anti-NMDAR encephalitis group than that of the controls. (D-E) CD44 levels both in the serum (E, p<0.0001) and the CSF (D, p=0.018) were significantly higher in the anti-NMDAR encephalitis group, compared with the control group. Differences in CSF sTREM2 level, serum sTREM2 level, serum MMP9, and CSF CD44 were assessed by the Mann-Whitney test between the anti-NMDAR encephalitis group and the control group. Differences in serum CD44 levels were assessed by the independent two-sample t-test.
Figure 2

Correlation analysis of sTRME2 levels in CSF or serum with other parameters.

(A-C). The CSF sTREM2 level is positively related to the serum sTREM2 level ($r=0.426, p=0.021$), the CSF CD44 level ($r=0.702, p<0.0001$), and the serum MMP9 level ($r=0.428, p=0.021$). (D-F). The CSF sTRME2 level is positively related to mRS scale ($r=0.422, p=0.023$) and age ($r=0.407, p=0.028$), while is negatively related to GCS score ($r=-0.401, p=0.031$). Coefficients were assessed by the Spearman correlation analysis.
Figure 3

Correlation analysis of CSF CD44 levels with clinical parameters and serum MMP9 levels.

(A-B) The CSF CD44 level is positively related to the mRS scale ($r=0.509$, $p=0.005$) and negatively related to the GCS score ($r=-0.382$, $p=0.041$). (C) The CSF CD44 level is positively related to the serum MMP9 level ($r=0.467$, $p=0.011$). (D) The serum MMP9 level is positively related to the Qalb value ($r=0.493$, $p=0.007$). Coefficients were assessed by the Spearman correlation analysis.