Impact of blood-brain barrier disruption on newly diagnosed neuromyelitis optica spectrum disorder symptoms and prognosis

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Background: Blood-brain barrier (BBB) disruption and ensuing immune activation are central to the pathogenesis of central nervous system (CNS) inflammatory diseases. However, the influence of BBB permeability on the clinical signs and prognosis of newly diagnosed neuromyelitis optica spectrum disorder (NMOSD) has not been examined. We investigate the relationships between BBB permeability as showed by the albumin quotient (qalb) and clinical features of NMOSD.

Methods: Demographic and clinical data of 46 patients, including peripheral blood (PB) measures (serum albumin concentration and total leukocyte, neutrophil, total lymphocyte, CD4+ T cell, and CD8+ T cell counts, complement C3 and C4 concentrations, AQP4-IgG titer),autoimmune antibody titers (ANA/SSA/ SSB/Ro-52), and cerebrospinal fluid (CSF) parameters (total leukocyte count, total protein and albumin concentrations, AQP4-IgG titer), were compared between qalb(BBB permeability) increased and normal groups. Complete measures were not obtained from 9 patients, but all other measures were included in the analysis.

Results: According to the calculated qalb, 15 patients with albumin quotient (qalb) > $(4 + age/15) \times 10{-}3$ were assigned to the qalb increased (high BBB permeability) group (33%) and the remainder to the qalb normal group. Compared to the qalb normal group, the qalb increased group exhibited significantly lower serum albumin (P=0.001) and CD4+ T cell count (P=0.044), CD8+ T cell count (P=0.014), and total T lymphocyte count (P=0.016). The qalb increased group proved higher CSF albumin, total protein, leukocyte count, and IgG titer (all P=0.000). Optic neuritis and optic nerve abnormalities on magnetic resonance images were also more frequent in the qalb increased group (P=0.037 and 0.038, respectively). Patients in the qalb increased group showed significantly poorer treatment response as indicated by the lower post-treatment change in Expanded Disability Status Scale (EDSS) score compared to the qalb normal group.

Conclusions: BBB permeability is strongly associated with the clinical features and treatment response of newly diagnosed NMOSD. The qalb is a potentially valuable indicator of disease severity and an index to guide personalized treatment.

Keywords: Blood-brain barrier (BBB); neuromyelitis optic spectrum disorders; newly diagnosis; optic neuritis; albumin quotient (qalb)

Introduction

Aquaporin-4 antibody (AQP4-IgG) is a specific NMOSD marker and the discovery of AQP4-IgG in NMOSD patients is the first clue for pathogenesis of disease. Neuromyelitis optica spectrum disorder (NMOSD) is an autoimmune-mediated inflammatory demyelinating disease with relapsing-remitting tendency. Notably, AQP4-IgG may persist in serum for many years, even with the absence of symptoms (1); therefore, the pathogenesis of NMOSD is still not fully understood.

Adding onto this, It is currently believed that the central pathogenic event in NMOSD is the entry of AQP4-IgG into the CNS through a damaged blood-brain barrier (BBB), triggering complement activation and granulocyte infiltration leading to astrocyte death (2). At the cellular level, AQP4 is expressed in astrocyte foot processes, with particularly abundant expression in the optic nerve, spinal cord, brainstem, hypothalamus, and cerebral ventricles, where it serves to maintain water balance by regulating the circulation of intracellular and extracellular water (3).

In AQP4-IgG seropositive rats, disruption of the BBB using pulsed ultrasound-induced NMO-like lesions in the brain and spinal cord by allowing entry of serum IgG. Central nervous system (CNS) inflammation, demyelination, and astrocyte damage are the primary pathological manifestations of NMO (4), and aberrant BBB permeability is associated with greater Extended Dysfunction Status Scale (EDSS) score and spinal cord lesion length (5). Moreover, BBB permeability, as assessed by the qalb, is strongly related to the degree of disability and brain atrophy after MS onset (6). In NMO spectrum disorder (NMOSD) as well, albumin quotient (qalb) is associated with the degree of motor disability (7).

However, there have been few studies on NMOSD in the acute phase, so there is a dearth of clinical and prognostic data on this patient population. Given the importance of BBB permeability in NMOSD pathogenesis, a convenient and straightforward biomarker is needed to evaluate disease severity and treatment outcome, predict recurrence, and guide individualized treatment decisions in newly diagnosed NMOSD patients. Therefore, we compared multiple peripheral types of blood and cerebrospinal fluid (CSF) metrics, magnetic resonance imaging (MRI) signs, and EDSS scores between NMOSD patients with high or normal BBB permeability as assessed by the qalb. The primary aim of this investigation is to determine whether higher BBB permeability during the acute phase of NMOSD is associated with greater symptom severity and lower post-treatment change.

Methods

Ethics statements

The Ethics Committee approved the study of the First Affiliated Hospital of Guangxi Medical University.

Study population

We included 46 NMOSD patients admitted to the Department of Neurology, First Affiliated Hospital of Guangxi Medical University from January 2017 to March 2019. The cohort included 4 males and 42 females ranging from 14 to 78 years old. Age at onset was 42.9±15.5 years, and the average disease duration was 1.14±1.05 months. Symptoms gradually increased following onset. All patients were diagnosed based on the 2015 NMOSD diagnostic criteria developed by the International Panel for NMO Diagnosis (IPND). All patients provided informed consent to blood and CSF sampling by venipuncture and lumbar puncture, respectively. Exclusion criteria were treatment with methylprednisolone, gamma globulin, or plasma replacement before hospitalization, or diseases such as myasthenia gravis, meningitis, brain abscess, and epilepsy that may affect qalb index and EDSS score. In this study, BBB permeability was assessed according to qalb = $(4 + age/15) \times 10^{-3}$, increased BBB permeability was defined as qalb > $(4 + Age/15) \times 10^{-3}$ (8). And all tests and qalb index calculations were performed before treatment.

Treatment

All patients with newly diagnosed NMOSD were treated with methylprednisolone 1.0 g/day for 3 days.

Expanded disability status scale score

The EDSS score was used to assess the severity of symptoms before and after treatment. An experienced neurologist performed the entire evaluation procedure.

MRI images

All patients were examined by T1-weighted, T2-weighted, and T2-Flair imaging of the optic nerve, brain, cervical

Table 1 Clinical features

Clinical features	Qalb normal (n=31)	Qalb increased (n=15)	χ^2	t	p
No. of males (%)	4/31 (13%)	0/15 (0%)	1.863		0.172
Age at onset, years	45.85±16.33	39.45±12.44		−1.500	
Disease duration, month	1.05±1.04	1.33±1.03		0.824	
Onset symptoms (%)					
ON onset	4/31 (13%)	6/15 (40%)	4.343		0.037
Brain symptom onset	2/31 (6%)	2/15 (13%)	0.603		0.437
Sensory or motor onset	28/31 (90%)	14/15 (93%)	0.115		0.734
AQP4-IgG (%)					
Sero AQP4-IgG+	20/31 (65%)	7/15 (47%)	1.328		0.249
CSF AQP4-IgG+	18/31 (58%)	6/15 (40%)	1.322		0.250
MRI abnormality (%)					
Optic nerve	0/31 (0%)	2/15 (13%)	4.321		0.038
Brain	14/31 (45%)	7/15 (47%)	0.009		0.923
Spinal cord	26/31 (84%)	11/15 (73%)	0.713		0.398
Autoimmune antibody (%)	11/31 (35%)	7/14 (50%)	2.729		0.099

Qalb, albumin quotient; ON, optic neuritis.

spinal cord, and thoracic spinal cord using a 1.5-T MRI system. An experienced neuroradiologist reviewed the images.

AQP4-IgG analysis

According to the latest guidelines, AQP4-IgG was detected by indirect immunofluorescence based on cell transfection.

Peripheral blood (PB) and CSF analysis

Total leukocyte, neutrophil, CD4+ T cell, CD8+ T cell, and total T lymphocyte counts, as well as albumin, complement C3, complement C4, and AQP4-IgG titer, autoimmune antibody (ANA/SSA/SSB/Ro-52) concentrations, were measured from serum samples. In addition, total leukocyte count and total protein, albumin, and AQP4-IgG concentrations were measured from CSF samples. Measures of CD4+ T cells, CD8+ T cells, complement C3, and C4, and ANA/SSA/SSB/Ro-52 were not all conducted in nine patients, but all other measures were included in the analysis.

Statistical analysis

All statistical analyses were performed using SPSS

24.0 software. Categorical data are expressed as ratios and compared between groups by the chi-square test. Continuous data with normal distributions are expressed as $\bar{\chi}$±S and compared between groups by independent samples *t*-test. Continuous data with non-normal distributions are expressed as the median [interquartile range] and compared between groups by Mann-Whitney U-test. A P<0.05 (two-tailed) is considered significant for all tests.

Results

Clinical features

Patients were divided into high and normal BBB permeability groups according to the qalb, with 15/46 patients (33%) in the qalb increased group [qalb > (4 + age/15) × 10^{-3}] and the remainder in the qalb normal group [qalb ≤ (4 + age/15) × 10^{-3}] (*Table 1*).

Increased BBB permeability increased the probability of optic neuritis

The probability of patients with optic neuritis was significantly higher in the qalb increased group (P=0.037). Similarly, the probability of patients with abnormal optic nerve MR imaging manifestations on MR images was higher in the qalb increased group (P=0.038) (*Figure 1*).
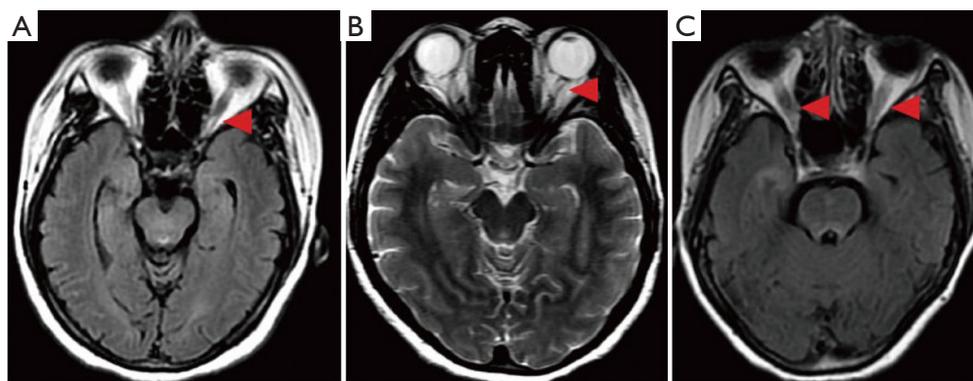
Figure 1 Abnormal optic nerves MR images in the qalb increased group. (A,B) Abnormal signal of the left nerve, (C) bilateral optic nerves thickening. MR, magnetic resonance; Qalb, albumin quotient.
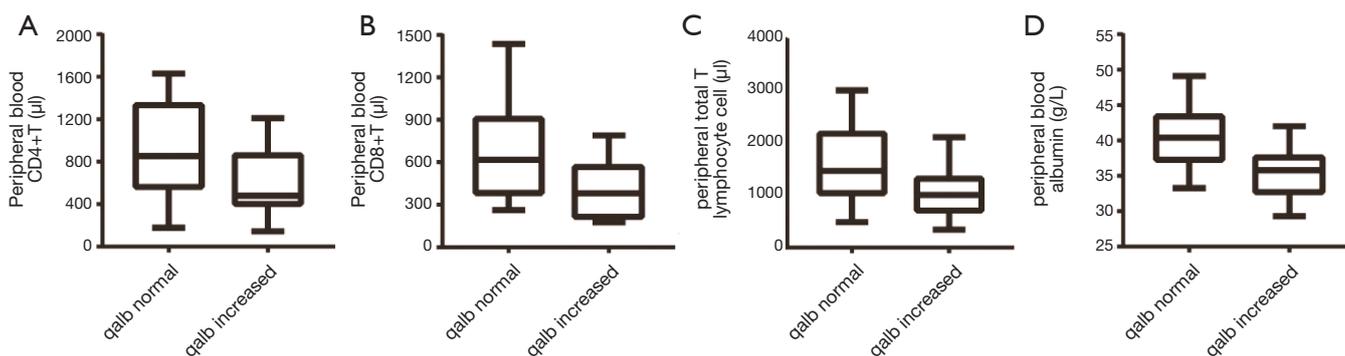
Figure 2 CD4+T/CD8+T/peripheral blood total T lymphocytes/peripheral blood albumin were lower in the qalb increased group than in the qalb normal group (P=0.044, 0.014, 0.016, 0.001). Qalb, albumin quotient.

BBB permeability and PB abnormalities

Compared to the qalb normal group, the qalb increased group demonstrated no significant differences in total leukocyte count (P=0.200), neutrophil count (P=0.761), total lymphocyte count (P=0.679), complement C3 concentration (P=0.705), and complement C4 concentration (P=0.306). However, the qalb increased group exhibited significantly lower serum albumin (P=0.001) and CD4+ T cell count (P=0.044), CD8+ T cell count (P=0.014), and total T lymphocyte count (P=0.016) (*Figure 2*).

BBB permeability and CSF abnormalities

The total number of leukocytes in the CSF was significantly elevated in the qalb increased group (P=0.007). Similarly, the qalb increased group demonstrated elevated CSF albumin, IgG, and total protein (all P=0.000) (*Figure 3*).

BBB permeability was associated with poorer treatment response

Patients in the qalb normal group showed better symptom relief following methylprednisolone treatment than the qalb increased group, as indicated by the change in the EDSS score (P=0.028) (*Table 2*).

Discussion

While several previous studies have explored the relationship between qalb and NMOSD's clinical characteristics, it is unclear the effect of qalb on newly diagnosed NMOSD. We found that BBB disruption, as evidenced by high qalb, is associated with optic nerve
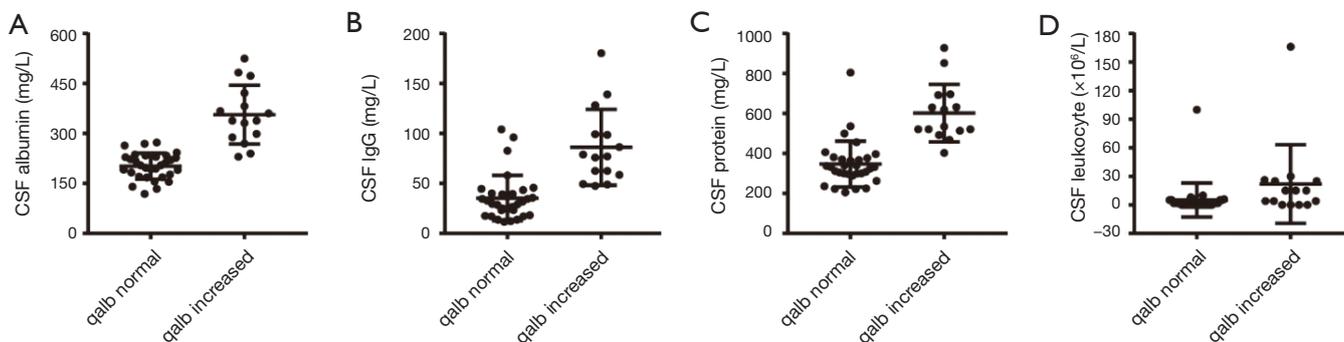
Figure 3 CSF albumin/IgG/protein/leukocyte were higher in the qalb increased group than in the qalb normal group (P=0.000, 0.000, 0.000, 0.007). CSF, cerebrospinal fluid; Qalb, albumin quotient.

Table 2 EDSS scale (before and after treatment)

EDSS scale	Qalb normal (n=31)	Qalb increased (n=15)	P
EDSS scale before treatment	4.11±2.20	3.70±2.27	0.431
EDSS scale after treatment	3.39±2.38	3.47±2.45	0.759
EDSS D-value	0.73±0.84	0.23±0.56	0.028

EDSS, Expanded Disability Status Scale; Qalb, albumin quotient; D-value, difference value.

abnormalities on MR images, poorer treatment response, lower CD4+ and CD8+ T cells in peripheral blood, and elevated total protein and leukocytes in CSF. Collectively, these blood and CSF changes are consistent with increased BBB permeability as a significant factor influencing NMOSD severity and treatment response in the acute phase.

Characteristics of the CNS microvasculature confer the BBB, and under normal circumstances, provides a continuous barrier against peripheral cells (such as leukocytes), proteins, and other factors. Furthermore, BBB component cells express multiple transport pathways that can regulate the exchange of materials between the PB and the CNS. This selective barrier function is critical for maintaining the optimal extracellular microenvironment for neuronal signaling in the CNS. The BBB also protects the CNS from peripheral inflammatory factors and cells as well as viruses, bacteria, and bacterial toxins. Disruption of BBB integrity allows these factors, including immunocytes and pro-inflammatory cytokines, to access vulnerable neuronal populations, ultimately inducing neuroinflammatory diseases (9). Astrocytes are a critical component of the BBB and regulate barrier function (10). In addition, astrocytes couple neuronal activity to local blood flow thus mediating homeostatic regulation of neuronal metabolism (11). Astrocytes also promote angiogenesis by secreting angiopoietin 1 (Ang 1) and altering BBB permeability by regulating junctional protein expression (12). Aquaporin 4 (AQP4) is highly expressed in astrocyte foot processes that abut the neurovasculature, with particularly high expression in the brainstem, optic nerve, spinal cord, and periventricular regions (13), where it functions mainly to maintain water homeostasis. These regions of high AQP4 expressional are also the sites of typical NMO lesions (14), strongly implicating AQP4 in disease pathogenesis. Indeed, it is believed that under BBB degradation, AQP4-IgG invades the CNS and binds to AQP4 on astrocytes, triggering an immune cascade that ultimately leads to astrocyte death, loss of extracellular and metabolic homeostasis, and further BBB degradation (13).

However, nearly one-third of NMO patients are seronegative for AQP4-IgG (14). Therefore, AQP4-IgG titer provides no prognostic information in these cases. Alternatively, our results suggest that qalb can be used for the prognosis of all NMOSD patients. We report that BBB disruption leads to worse prognosis in NMOSD, by allowing pro-inflammatory factors, leukocytes, and AQP4 antibodies to invade the retinal or CNS parenchyma, resulting in

neuroinflammation. Our findings are in accord with previous reports that qalb is associated with CSF albumin, CSF leukocyte count, and spinal cord lesion length (5). In addition, patients with increased qalb demonstrated a higher probability of developing optic neuritis, a finding not previously reported. Retinal nerve fiber layer (RNFL) loss is more severe in NMO than in MS according to optical coherence tomography (OCT) (15). The onset of optic neuritis in NMOSD is believed to result from the entry of T cells into the CNS through the disrupted BBB. During optic neuritis, the retinal nerve fiber/ganglion cell layer often becomes thinner (16), suggesting damage to the optic nerve and retina (17). T cells can enter the retina through two pathways, the vascular pathway of the nerve fiber/ganglion cell/inner plexus layer or the outer nucleus/ outer plexus layer/core layer, AQP4-IgG, and complement resulting in the loss of AQP4 and destruction of Müller glial cells (18). This phenomenon has also been described in the autopsy results of NMOSD patients (19).

The mechanism of CD4+ T and CD8+ T cell reduction in PB of NMO is complex. OX40 (CD134), a member of the tumor necrosis factor (TNF) receptor family expressed primarily on activated CD4+ T and CD8+ T cells, was found to be downregulated in PB of NMO patients (20). Further, both the current results and a previous study (21) have found lower ratio PB CD4+ T cells and CD8+T cells, suggesting that BBB disruption is associated with a peripheral blood lymphocyte (PBL) imbalance. Based on earlier studies, we speculate that this reduction is related to the disruption of immune homeostasis. Linhares *et al.* (22) reported enhanced cell death in PHA-activated cultures from NMO patients and low release of interleukin-2 (IL-2) and interferon-gamma (INF-γ). This lowered release of IL-2 and IFN-γ may trigger activation-induced cell death (AICD) of lymphocytes (23). Another potential mechanism is cell differentiation. Both T-helper cell 17 (Th17) and the cytokine IL-17A are reported to be abnormally increased in the PB of NMO patients. The resulting elevation in transforming growth factor-β, IL-6, and IL-21 in PB promotes the differentiation of CD4+ T cells and the secretion of cytokines. Abnormally high Th17 cell numbers elevated IL-17 secreted by CD8+ T cells, and higher IL-17A are all detected in NMO (24). In addition, Th17 cell number and IL-17A concentration are correlated with the EDSS score (25). However, we were unable to directly study this potential pathway due to an inability to detect CD4+ T cell subsets and related cytokines. Future studies are planned to assess this mechanism. Leukocytes were also

significantly elevated in the CSF of patients with newly diagnosed NMOSD, especially the increased qalb group, in accord to a previous study by Wang *et al.* (5). We suspect that this phenomenon is related to cell migration.

Thus, the destruction of the BBB in NMOSD results in the immune invasion, which triggers the release of pro-inflammatory cytokines and complement factors from astrocytes resulting in further leukocytes infiltration (especially eosinophils and neutrophils) during the period of disease deterioration (14). This study also proves the utility of qalb as a biomarker for evaluating BBB permeability and for the prognosis of newly diagnosed NMOSD. Detection of high qalb may help guide individualized treatment.

There are limitations to this study. All patients have undergone only one lumbar puncture and only one qalb has been measured, while the effects of BBB permeability on NMOSD pathogenesis need to be confirmed by multiple lumbar punctures performed at different stages.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form and declare: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The Ethics Committee approved the study of the First Affiliated Hospital of Guangxi Medical University [No. 2019(KY-E-119)].

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