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RESEARCH ARTICLE

Serum levels of cell adhesion molecules in patients with neuromyelitis optica spectrum disorder

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Abstract

Objectives: Blood–brain barrier (BBB) disruption is a critical pathological process involved in neuromyelitis optica spectrum disorder (NMOSD). Here, we characterized the profile of five cell adhesion molecules in patients with NMOSD. **Methods**: We measured levels of cell adhesion molecules, including ICAM-1, ICAM-2, VCAM-1, PECAM-1, and NCAM-1, in the serum of 28 patients with NMOSD, 24 patients with multiple sclerosis (MS), and 25 healthy controls (HCs). **Results**: ICAM-2 levels (median: 394.8 ng/mL) were increased in patients with NMOSD compared with MS (267.1 ng/mL, $P = 0.005$) and HCs (257.4 ng/mL, $P = 0.007$), and VCAM-1 and ICAM-1 levels were higher in patients with NMOSD (641.9 ng/mL and 212.7 ng/mL, respectively) compared with HCs (465 ng/mL [$P = 0.013$] and 141.8 ng/mL [$P = 0.002$], respectively). However, serum PECAM-1 levels were lower in patients with NMOSD (89.62 ng/mL) compared with MS (106.9 ng/mL, $P = 0.015$) and HCs (107.2 ng/mL, $P = 0.007$). Receiver operating characteristic curve analysis revealed that PECAM-1 (area under the curve (AUC): 0.729) and ICAM-2 (AUC: 0.747) had adequate abilities to distinguish NMOSD from MS, and VCAM-1 (AUC: 0.719), PECAM-1 (area under the curve: 0.743), ICAM-1 (AUC: 0.778), and ICAM-2 (AUC: 0.749) exhibited potential to differentiate NMOSD and HCs. Serum levels of PECAM-1 also demonstrated a negative correlation with Kurtzke Expanded Disability Status Scale scores in patients with NMOSD. **Interpretation**: Our results reveal possible BBB breakdown signals specifically observed in NMOSD and highlight the potential role of cell adhesion molecules as biomarkers of this disease.

Introduction

Neuromyelitis optica spectrum disorder (NMOSD) and multiple sclerosis (MS) are immune-mediated neuroinflammatory diseases of the central nervous system (CNS). Clinically differentiating these two diseases is critical because their therapeutic regimens vary and a few medications for MS may exacerbate NMOSD.[1] Following the discovery of anti–aquaporin 4 antibody (AQP4-IgG) in patients with NMOSD, clinicians now consider these two diseases to be distinct entities with potentially different pathomechanisms.[2-4] AQP4 is a water channel protein primarily expressed in astrocyte foot processes, which form the glia limitans of the blood–brain barrier (BBB).[5] Circulating AQP4-IgGs enter the brain through BBB leakage and initiate complement- and antibody-dependent cytotoxic cascades by binding to AQP4, which leads to profound infiltration of lymphocytes, macrophages, and eosinophils as well as extensive destruction in the diencephalon, periependymal regions, area postrema, and spinal cords in patients with NMOSD.[6,7] AQP4-IgG is considered a highly specific biomarker for NMOSD[5]; however, 20–30% of patients with NMOSD do not exhibit AQP4-IgGs, which hampers efforts to distinguish NMOSD from MS in these patients. Therefore, other biomarkers

are required for discriminating between NMOSD and MS.

BBB disruption is a hallmark of NMOSD that has been demonstrated in magnetic resonance imaging (MRI) with gadolinium contrast-enhanced lesions, particularly in the acute stage of NMOSD.[6,8,9] BBB destruction is essential for AQP4-IgGs to enter the CNS.[9-11] BBB breakdown regulates the expression of endothelial cell adhesion molecules that tightly mediate immune cell extravasation and trafficking into the CNS as well as contribute to modulation of vascular integrity.[12,13] Endothelial cell adhesion molecules such as vascular cell adhesion molecule-1 (VCAM1), intracellular adhesion molecule-1 (ICAM1), intracellular adhesion molecule-2 (ICAM2), platelet endothelial cell adhesion molecule-1 (PECAM1), and neural cell adhesion molecule-1 (NCAM1) belong to the immunoglobulin superfamily.[14] Studies have suggested that VCAM1, ICAM1, and PECAM1 are instrumental in BBB disruption for transmigration of activated peripheral lymphocytic cells to the CNS.[6,12,13,15]

In MRI, NMOSD is characterized by longitudinally extensive gadolinium contrast-enhanced lesions at the spinal cord and/or optic nerves, whereas periventricular plaques with partial ring enhancement are frequently observed in MS.[16] The differences in patterns and distributions of contrast-enhanced lesions suggest these two diseases generate different BBB breakdown patterns. The discovery of BBB-reactive antibodies, such as GRP78 antibodies in NMOSD and galactin-3 antibodies in MS, further implies that the BBB breakdown mechanisms differ between these two neuroinflammatory diseases.[6,16] Although the exact role of BBB breakdown in the pathogenesis of NMOSD remains elusive, cell adhesion molecules may represent BBB function and serve as disease activity biomarkers for many neuroinflammatory diseases of the CNS.[5,9,17] Therefore, we evaluated the profile of five cell adhesion molecules, ICAM1, ICAM2, VCAM1, PECAM1, and NCAM1, in patients with NMOSD. The levels of these molecules were subsequently compared with those in patients with MS and healthy controls (HCs).

Materials and methods

Ethics approval and consent to participate

The collection of venous blood from enrolled patients was approved by the Institutional Review Board of Chang Gung Memorial Hospital (ethical license No: 201302260A3D001 and 201701423B0). Informed consents were obtained from all participants in this study.

Patient recruitment

This is a cross-sectional study from 1 January 2014, to 31 December 2019, in Chang Gung Memorial Hospital-Linkou Medical Center in Taiwan. Patients from the neurology ward diagnosed with NMOSD or MS by two experienced specialists in neuroimmunology (LS Ro and KH Chang) according to the international consensus diagnostic criteria for NMOSD[18] and the McDonald criteria,[19] respectively, were recruited. Both AQP-4 IgG positive and negative were eligible for the study. No participant had systemic infection, chronic renal failure, cardiac or liver dysfunction, malignancies, or autoimmune diseases other than NMOSD and MS. Healthy controls were recruited from neurology outpatient clinics by a convenience sample of individuals seen at the time of recruitment, and were frequency matched for sex and age of patients.

Sample collection

Venipunctures were performed on all participants. Blood samples of the patients with NMOSD and MS were collected within 2 weeks after symptom onset or acute relapses of the disease. All samples were obtained before treatment with corticosteroids, intravenous immunoglobulins, or plasmapheresis. Blood samples were maintained at room temperature for 30 min and then centrifuged at 1000–2000 *g* for 10 min. Serum was carefully collected from the supernatant, aliquoted, frozen at −80°C, and stored until analysis.

The neurological disability of the patients at the time of venipuncture was assessed using the Kurtzke Expanded Disability Status Scale (EDSS).[20]

Enzyme-linked immunosorbent assays for quantification of cell adhesion molecule expression in serum

We used enzyme-linked immunosorbent assay kits to evaluate the serum levels of VCAM1 (R&D), PECAM1 (R&D), NCAM1 (RayBio), ICAM1 (R&D), and ICAM2 (MyBioSource). Each assay was measured in duplicate for each sample at the same time.

Statistical analysis

Prism 8 (GraphPad) was used for statistical analyses. The D'Agostino–Pearson test[21,22] revealed that ICAM1, ICAM2, VCAM1, PECAM1, and NCAM1 levels were not normally distributed. Therefore, the Kruskal–Wallis test (nonparametric test to compare unmatched groups) was applied to compare the differences in these noncategorical variables among the NMOSD, MS, and HC groups. For the variables with significant differences among NMOSD,

MS, and HCs, namely ICAM1, ICAM2, VCAM1, and PECAM1, Spearman correlation was applied to evaluate the relationship between their levels and the EDSS at the time of sample collection. Data are expressed as median, interquartile range (IQR), and 95% confidence interval (CI). All *P* values were two-tailed, and $P < 0.05$ was considered significant. Variables with significant differences among NMOSD, MS, and HCs were further analyzed using receiver operating characteristic (ROC) curves. Area under the ROC curve (AUC) analysis was applied for these four molecules to measure their usefulness for distinguishing those with NMOSD from those with MS and those with NMOSD from HCs. An AUC value ≥0.7 was considered suitable to distinguish groups.

Results

The demographic data of all groups were summarized (Table 1). We recruited 28 patients with NMOSD (23 women, 5 men) and 24 patients with MS (16 women, 8 men), as well as 25 sex- and age-matched HCs (17 women, 8 men). Among those with NMOSD, 26 patients were AQP4-IgG positive and two were negative. Patients with NMOSD exhibited significantly higher EDSS scores (4.27 ± 1.90) than did those with MS (3.31 ± 1.43, $P = 0.040$). In total, 20 (72.43%) patients with NMOSD presented relapses of longitudinally extensive spinal cord lesions, and optic neuritis and area postrema syndrome were observed in six (21.43%) and two (7.14%) patients, respectively. ICAM1 levels in serum were significantly higher in patients with NMOSD (median: 212.7 ng/mL, IQR: 154.2–299.9 ng/mL, 95% CI: 192.3–270.5, Fig. 1A) than in HCs (median 141.8 ng/mL, IQR: 118.4–187.5 ng/mL, 95% CI: 116–163.8, $P = 0.002$) but not different from those with MS (median 154.3 ng/mL, IQR: 121.6–217 ng/mL, 95% CI: 142–189.9). Patients with NMOSD exhibited higher serum levels of ICAM2 (median: 394.8 ng/mL, IQR: 286.5–551 ng/mL, 95% CI: 360.4–495.6, Fig. 1B) than did those with MS (median: 267.1 ng/mL, IQR: 176.3–411.3 ng/mL, 95% CI: 230.6–337.1, $P = 0.005$) and HCs (median: 257.4 ng/mL, IQR: 215.2–397.7 ng/mL, 95% CI: 237.4–337.6, $P = 0.007$). Patients with NMOSD exhibited higher serum levels of VCAM1 (median: 641.9 ng/mL, IQR: 433.5–1326 ng/mL, 95% CI: 702.4–1303, Fig. 1C) than did those with MS (median: 499 ng/mL, IQR: 392.3–605.5 ng/mL, 95% CI: 454.3–567.1, $P = 0.161$) and HCs (median: 465 ng/mL, IQR: 399.3–538.6 ng/mL, 95% CI: 430.4–506.4, $P = 0.013$). Serum levels of PECAM1 in patients with NMOSD (median: 89.62 ng/mL, IQR: 74.26–111.2 ng/mL, 95% CI: 82.4–99.62, Fig. 1D) were significantly lower than in those with MS (median: 106.9 ng/mL, IQR: 91.61–133.9 ng/mL, 95% CI: 103–130.3, $P = 0.015$) and HCs (median: 107.2 ng/mL, IQR: 98.41–122.8 ng/mL, 95% CI: 102.9–124.6, $P = 0.007$). Serum NCAM1 levels were similar between patients with NMOSD, patients with MS, and HCs (Fig. 1E).

AUC analysis revealed that ICAM2 (AUC = 0.747, 95% CI: 0.615–0.88, $P = 0.02$, Fig. 2C) and PECAM1 (AUC = 0.729, 95% CI: 0.595–0.864, $P = 0.005$, Fig. 2G) have strong abilities to distinguish patients with NMOSD from those with MS, and ICAM1 (AUC = 0.777, 95% CI: 0.654–0.901, $P = 0.001$, Fig. 2B), ICAM2 (AUC = 0.749, 95% CI: 0.617–0.88, $P = 0.002$, Fig. 2D), VCAM1 (AUC = 0.719, 95% CI: 0.578–0.859, $P = 0.006$, Fig. 2F) and PECAM1 (AUC = 0.743, 95% CI: 0.608–0.878, $P = 0.003$, Fig. 2H) demonstrated adequate potential to distinguish patients with NMOSD from HCs. We further analyzed the correlation between the serum levels of ICAM1, ICAM2, VCAM1, or PECAM1 and disease severity, which was evaluated using the EDSS at sample collection (Fig. 3). The results revealed a significant correlation between serum levels of PECAM1 and EDSS score in patients with NMOSD ($r = -0.569$, $P = 0.002$, Fig. 3D). However, serum levels of ICAM1 ($r = -0.088$, Fig. 3A), ICAM2 ($r = -0.016$, Fig. 3B), and VCAM1 ($r = -0.045$, Fig. 3C) did not demonstrate correlations with EDSS score.

Discussion

The immunoglobulin superfamily of cell adhesion molecules, including ICAM1, ICAM2, VCAM1, PECAM1, and

Table 1. Clinical characteristics of the patients with NMOSD, MS, and HCs

Parameter	NMOSD (n = 28)	MS (n = 24)	HC (n = 25)
Sex (female/male)	23/5	16/8	17/8
Age (years)	47.86 ± 15.16	39.96 ± 14.51	47.96 ± 14.09
Age at onset (years)	45.82 ± 13.59	37.83 ± 13.81	
EDSS	4.27 ± 1.90*	3.31 ± 1.43	
LESCL (%)	20 (72.43)	0 (0)	
ON (%)	6 (21.43)	2 (8.33)	
APS (%)	2 (7.14)	0 (0)	
AQP4-IgG (%)	26 (92.86)	0 (0)	
Lesion(s) with gadolinium enhancement (%)	23 (82.14)	19 (79.17)	

All blood samples were collected from patients with NMOSD and MS within 2 weeks after symptom onset or acute relapse of disease and before their treatment with corticosteroids, intravenous immunoglobulins, or plasmapheresis.

APS: area postrema syndrome; AQP4-IgG: antiaquaporin 4 antibody; EDSS: Kurtzke Expanded Disability Status Scale; HC: healthy control; LESCL: longitudinally extensive spinal cord lesion; MS: multiple sclerosis; NMOSD: neuromyelitis optica spectrum disorder; ON: optic neuritis.

*Statistically significant difference compared with MS, $P < 0.05$.

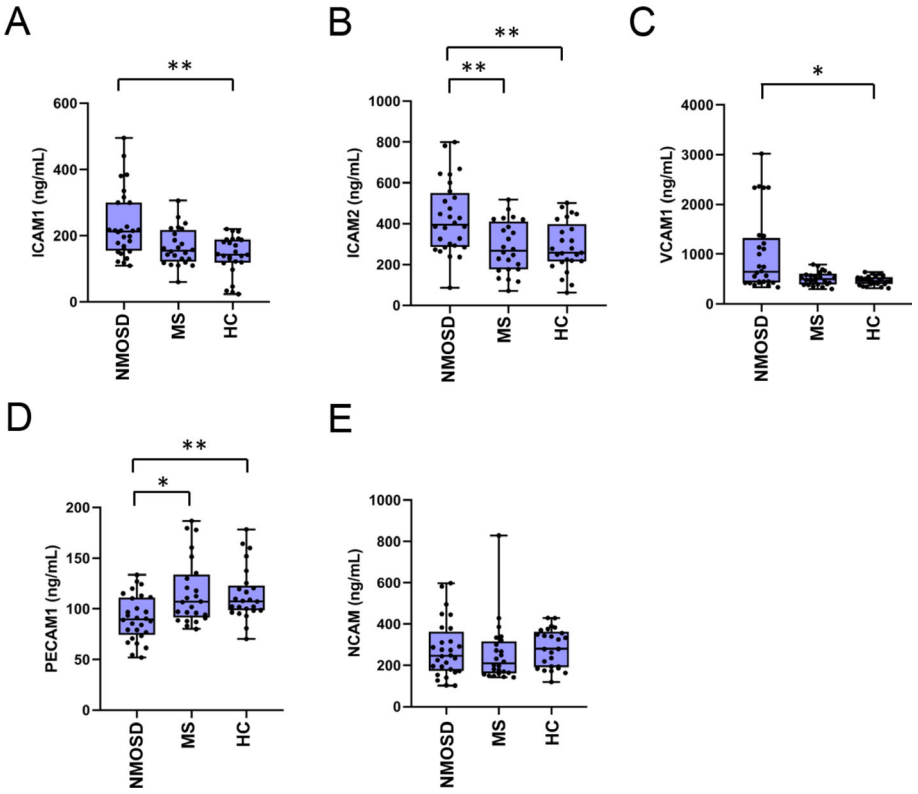

Figure 1. Serum levels of (A) ICAM1, (B) ICAM2, (C) VCAM1, (D) PECAM1, and (E) NCAM in patients with neuromyelitis optica spectrum disorder (NMOSD, n = 28), multiple sclerosis (MS, n = 24), and healthy controls (HCs, n = 25). Box-whisker plots depict the median and interquartile range (IQR) of each group. Error bars represent 95% confidence intervals. Statistically significant differences between two groups: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

NCAM1, are constitutional proteins expressed on endothelial cells, leukocytes, epithelial cells, and fibroblasts when the BBB is intact.[12,17] These cell adhesion molecules, which are upregulated by proinflammatory factors,[6,12,13] play essential roles in leukocyte–endothelial interaction and regulation of the neuroinflammatory cascade of immune-mediated CNS diseases by modulating vascular permeability and BBB integrity.[23] Increases of cell adhesion molecules in cerebrospinal fluid (CSF) or shedding into serum may be associated with BBB damage.[13] The dysregulation of cell adhesion molecules and disruption of the BBB lead to penetration of inflammatory cells and immunoglobulins into the CNS.[6,12,24] Patients with NMOSD exhibited a significant increase in serum ICAM2 compared with those with MS and HCs, an increase in serum VCAM1 and ICAM1 compared with HCs, but a significant decrease in serum PECAM1, with a negative correlation to disease severity. These markers also demonstrated potential for discriminating NMOSD from MS and HCs, suggesting these molecules might be candidate biomarkers for NMOSD.

Increased expression of blood ICAM1 in patients with NMOSD was reported by Uzawa et al.[9] However, alterations in PECAM1 and ICAM2 levels in NMOSD have not been reported. PECAM1 in blood is expressed on platelets, neutrophils, monocytes, and selected lymphocyte subsets and abundantly in the endothelial cells of intercellular junctions.[25] PECAM1 regulates BBB permeability, immune cell trafficking, and vascular remodeling.[23,25-27] It also exhibits pleiotropic action in both pro- and anti-inflammatory signaling pathways.[23] PECAM1 facilitates leukocyte transendothelial migration[28,29] and transduces mechanical signals in endothelial cells responding to fluid shear stress change by activating the proinflammatory transcription factor NF-κB.[23,30] However, PECAM1 also increases the threshold of leukocyte activation by recruiting inhibitory phosphatases to immunoreceptor tyrosine-based inhibitory motifs,[31-33] diminishes production of proinflammatory cytokines following exposures to endotoxins, and maintains vascular barrier integrity.[34,35] Studies have reported that PECAM1 is involved in the pathogenesis of MS, with higher PECAM1 levels observed in those with MS.[25,36-38] In our study, the low PECAM1 level in patients with NMOSD contrasted with the levels of ICAM1, ICAM2, and

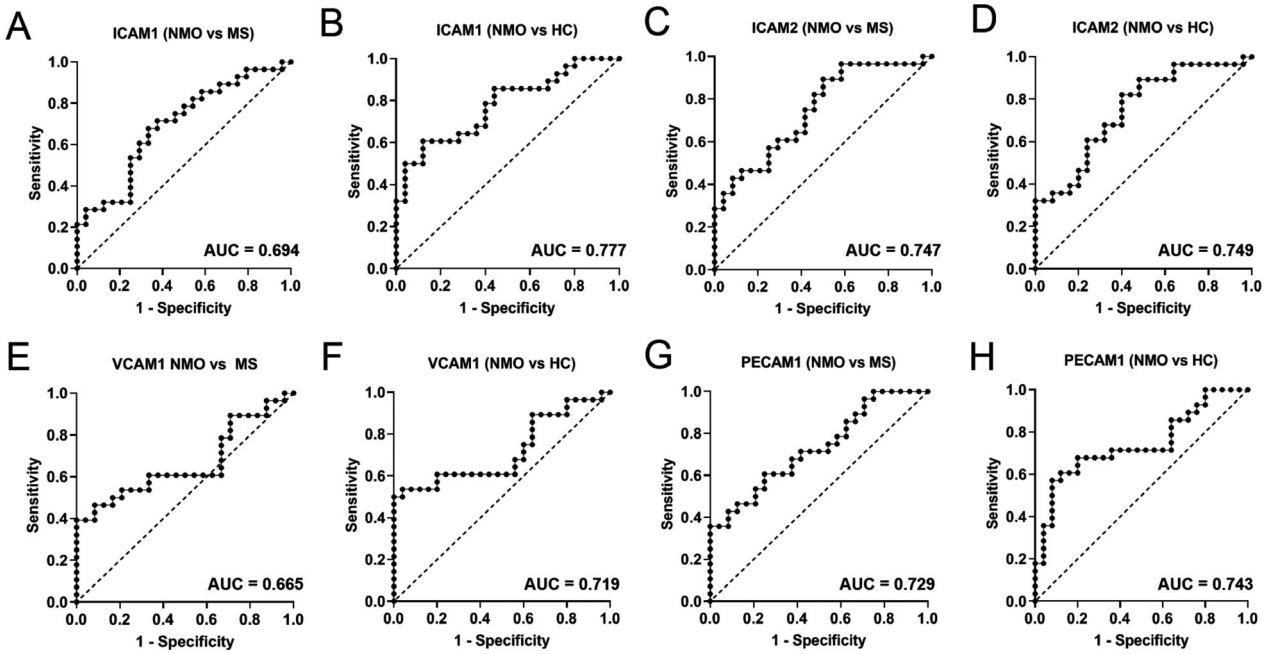

Figure 2. Receiver operating characteristic (ROC) curve for serum levels of ICAM1 (A-B), ICAM2 (C-D), VCAM1 (E-F), and PECAM1 (G-H) to distinguish patients with neuromyelitis optica spectrum disorder (NMOSD) from those with multiple sclerosis (MS) and healthy controls (HCs). AUC: area under the ROC curve

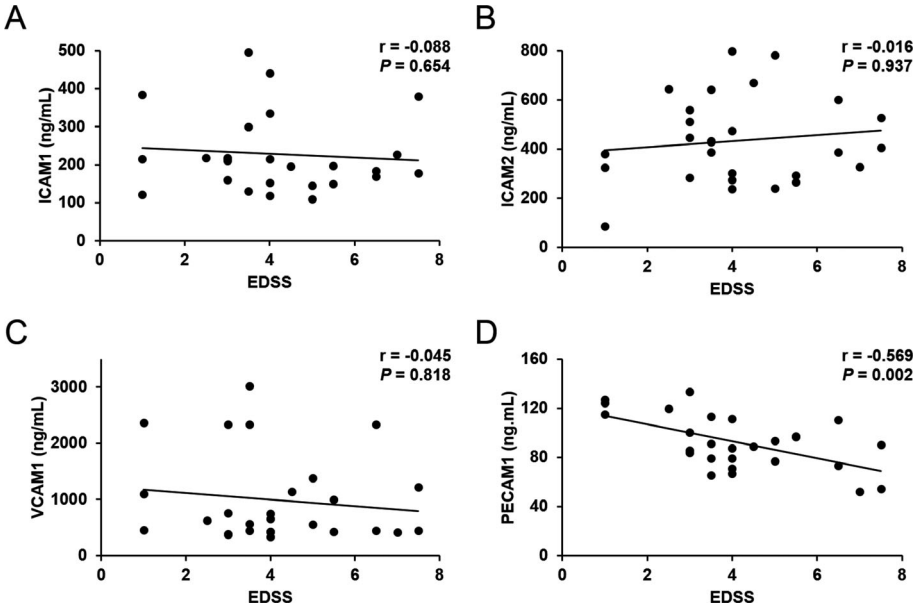

Figure 3. Correlations between serum levels of (A) ICAM1, (B) ICAM2, (C) VCAM1, or (D) PECAM1, and Kurtzke Expanded Disability Status Scale (EDSS) score in patients with neuromyelitis optica spectrum disorder (NMOSD). r: spearman correlation coefficient

VCAM1, suggesting that PECAM1 plays a different role from other cell adhesion molecules in NMOSD. The negative correlation between PECAM1 levels and EDSS further suggests the protective role of PECAM1 in the pathogenesis of NMOSD. ICAM2 is highly expressed on endothelial cells, platelets, and various leukocytes.[39,40] Endothelial ICAM2 is involved in the maturation of endothelial junctions and adhesion of leukocytes to the endothelium.[41] ICAM2 also contributes to neutrophil crawling and the initiation of paracellular diapedesis.[41,42]

Neutrophil binding to endothelial ICAM2 increases vascular permeability during acute neuroinflammatory processes.[43] The high level of ICAM2 in NMOSD suggests its role in BBB breakdown in NMOSD. The different levels of PECAM1 and ICAM2 evident between NMOSD and MS further indicate distinct immunopathogenesis of these two diseases.

By binding to α4 integrin/very late antigen-4 and leukocyte function–associated antigen 1, VCAM1 and ICAM1 promote transendothelial recruitment of immune cells and the subsequent neuroinflammatory cascade.[9,17,24] Uzawa et al. revealed that patients with NMOSD exhibit significantly higher CSF levels of ICAM1 and VCAM1 than do patients with MS.[9] Our results further revealed increased ICAM1 and VCAM1 serum levels in those with NMOSD compared with HCs. Consistent with Uzawa's findings,[9] serum levels of ICAM1 and VCAM1 in our study were not different between those with MS and HCs, implying that MS exhibits different mechanisms of BBB disruption and neuroinflammatory pathogenesis from NMOSD.

Studies have suggested that the involvement of NCAM1 in cell–cell adhesion, axonal fasciculation and outgrowth, synaptic plasticity, myelination, and remyelination has a role in the reparative mechanisms of myelin in MS.[44,45] An elevated NCAM1 level was also reported in MS.[45] However, none of the data were related to NMOSD. In the current study, we did not find significant changes of NCAM1 levels between patients with NMOSD, MS, and HCs. Studies have revealed that serum VCAM1, ICAM1, and PECAM1 levels are elevated in MS and correlate with gadolinium-enhanced MRI lesions.[36,37,46,47] By contrast, lower serum VCAM1 and PECAM1 levels in patients with optic neuritis who tend to develop MS were reported by Kalinowska-Lyszczarz et al.[17] We did not find significant differences in VCAM1, PECAM1, ICAM1, ICAM2, and NCAM1 levels between those with MS and HCs.

A possible explanation for the variable serum levels of these cell adhesion molecules among those with NMOSD, MS, and HCs is that these molecules might have different pro- or anti-inflammatory roles[23] in maintaining and regulating the vascular integrity of the BBB during neuroinflammatory processes. Another potential reason is that the immunopathogenesis of NMOSD and MS is distinct, leading to the involvement of different adhesion-induced signaling and BBB-reactive autoantibodies.[6,14]

This study had some limitations. The evolving diagnostic criteria for NMOSD and MS may explain the difference between our results and those of previous reports. Our sample size was also relatively small. The low number of AQP4-IgG-negative patients also limits the generalization of our results to this subpopulation of patients with NMOSD. Although not significantly different, the younger age in patients with MS in our study may have affected adhesion molecule levels. The CSF levels of these adhesion molecules were not available, and the details of their pathogenesis in NMOSD remained unknown. Nevertheless, our findings still highlight a new avenue in biomarker research in NMOSD.

Conclusions

In conclusion, our study demonstrated significantly higher levels of ICAM1, ICAM2, and VCAM1 and a lower level of PECAM1 in the serum of patients with NMOSD. These cell adhesion molecules could be potential biomarkers for distinguishing patients with NMOSD from those with MS and HCs. Furthermore, PECAM1 serum level could reflect clinical severity with a significantly negative correlation. These observations demonstrate a unique regulatory pattern of cell adhesion molecules in NMOSD.

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Conflicts of interest

None.

Author contribution

Kuo-Hsuan Chang and Long-Sun Ro contributed to conceptualization. Yen-Shi Lo and Bao-Luen Chang contributed to data curation. Kuo-Hsuan Chang and Bao-Luen Chang contributed to formal analysis. Kuo-Hsuan Chang contributed to funding acquisition. Kuo-Hsuan Chang and Bao-Luen Chang contributed to methodology. Kuo-Hsuan Chang, Long-Sun Ro, Chiung-Mei Chen, Rong-Kuo Lyu, Hong-Chou Kuo, Ming-Feng Liao, Chun-Wei Chang, Hong-Shiu Chang, Ching-Chang Huang, Yih-Ru Wu, Chun-Che Chu and Yi-Ching Weng contributed to resources. Kuo-Hsuan Chang contributed to

supervision. Bao-Luen Chang and Kuo-Hsuan Chang contributed to writing – original draft. Kuo-Hsuan Chang, Bao-Luen Chang and Long-Sun Ro contributed to writing – review & editing.

References

1. Eckstein C, Saidha S, Levy M. A differential diagnosis of central nervous system demyelination: beyond multiple sclerosis. J Neurol 2012;259(5):801–816.
2. Misu T, Fujihara K, Kakita A, et al. Loss of aquaporin 4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. Brain 2007;130(Pt 5):1224–1234.
3. Roemer SF, Parisi JE, Lennon VA, et al. Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. Brain 2007;130(Pt 5):1194–1205.
4. Lucchinetti CF, Mandler RN, McGavern D, et al. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. Brain 2002;125(Pt 7):1450–1461.
5. Melamed E, Levy M, Waters PJ, et al. Update on biomarkers in neuromyelitis optica. Neurol Neuroimmunol Neuroinflamm. 2015;2(4):e134.
6. Shimizu F, Nishihara H, Kanda T. Blood-brain barrier dysfunction in immuno-mediated neurological diseases. Immunol Med. 2018;41(3):120–128.
7. Papadopoulos MC, Verkman AS. Aquaporin 4 and neuromyelitis optica. Lancet Neurol. 2012;11(6):535–544.
8. Kim SM, Waters P, Vincent A, et al. Cerebrospinal fluid/serum gradient of IgG is associated with disability at acute attacks of neuromyelitis optica. J Neurol. 2011;258(12):2176–2180.
9. Uzawa A, Mori M, Masuda S, Kuwabara S. Markedly elevated soluble intercellular adhesion molecule 1, soluble vascular cell adhesion molecule 1 levels, and blood-brain barrier breakdown in neuromyelitis optica. Arch Neurol 2011;68(7):913–917.
10. Sharma R, Fischer MT, Bauer J, et al. Inflammation induced by innate immunity in the central nervous system leads to primary astrocyte dysfunction followed by demyelination. Acta Neuropathol 2010;120(2):223–236.
11. Bradl M, Misu T, Takahashi T, et al. Neuromyelitis optica: pathogenicity of patient immunoglobulin in vivo. Ann Neurol 2009;66(5):630–643.
12. Yang C, Hawkins KE, Dore S, Candelario-Jalil E. Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke. Am J Physiol Cell Physiol 2019;316(2):C135–C153.
13. Waubant E. Biomarkers indicative of blood-brain barrier disruption in multiple sclerosis. Dis Markers 2006;22(4):235–244.
14. van Buul JD, Hordijk PL. Endothelial signalling by Ig-like cell adhesion molecules. Transfus Clin Biol 2008;15(1–2):3–6.
15. Alvarez JI, Saint-Laurent O, Godschalk A, et al. Focal disturbances in the blood-brain barrier are associated with formation of neuroinflammatory lesions. Neurobiol Dis 2015;74:14–24.
16. Wildner P, Stasiolek M, Matysiak M. Differential diagnosis of multiple sclerosis and other inflammatory CNS diseases. Mult Scler Relat Disord 2019;37:101452.
17. Kalinowska-Lyszczarz A, Michalak S, Pawlak MA, et al. Serum sPECAM-1 and sVCAM-1 levels are associated with conversion to multiple sclerosis in patients with optic neuritis. J Neuroimmunol 2016;300:11–14.
18. Wingerchuk DM, Banwell B, Bennett JL, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology 2015;85(2):177–189.
19. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 2018;17(2):162–173.
20. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33(11):1444–1452.
21. D'Agostino RB. An omnibus test of normality for moderate and large size samples. Biometrika 1971;58(2):341–348.
22. D'Agostino R, Pearson ES. Tests for departure from normality. Empirical results for the distributions of b2 and $\sqrt{b}1$. Biometrika 1973;60(3):613–622.
23. Privratsky JR, Newman DK, Newman PJ. PECAM-1: conflicts of interest in inflammation. Life Sci 2010;87(3–4):69–82.
24. Ortiz GG, Pacheco-Moises FP, Macias-Islas MA, et al. Role of the blood-brain barrier in multiple sclerosis. Arch Med Res 2014;45(8):687–697.
25. Wimmer I, Tietz S, Nishihara H, et al. PECAM-1 stabilizes blood-brain barrier integrity and favors paracellular T-cell diapedesis across the blood-brain barrier during neuroinflammation. Front Immunol 2019;10:711.
26. Lertkiatmongkol P, Liao D, Mei H, et al. Endothelial functions of platelet/endothelial cell adhesion molecule-1 (CD31). Curr Opin Hematol 2016;23(3):253–259.
27. Kalinowska A, Losy J. PECAM-1, a key player in neuroinflammation. Eur J Neurol 2006;13(12):1284–1290.
28. Muller WA, Weigl SA, Deng X, Phillips DM. PECAM-1 is required for transendothelial migration of leukocytes. J Exp Med 1993;178(2):449–460.
29. Mamdouh Z, Chen X, Pierini LM, et al. Targeted recycling of PECAM from endothelial surface-connected compartments during diapedesis. Nature 2003;421(6924):748–753.
30. Tzima E, Irani-Tehrani M, Kiosses WB, et al. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature 2005;437(7057):426–431.

31. Newton-Nash DK, Newman PJ. A new role for platelet-endothelial cell adhesion molecule-1 (CD31): inhibition of TCR-mediated signal transduction. J Immunol 1999;163 (2):682–688.
32. Wong MX, Roberts D, Bartley PA, Jackson DE. Absence of platelet endothelial cell adhesion molecule-1 (CD31) leads to increased severity of local and systemic IgE-mediated anaphylaxis and modulation of mast cell activation. J Immunol 2002;168(12):6455–6462.
33. Rui Y, Liu X, Li N, et al. PECAM-1 ligation negatively regulates TLR4 signaling in macrophages. J Immunol 2007;179(11):7344–7351.
34. Carrithers M, Tandon S, Canosa S, et al. Enhanced susceptibility to endotoxic shock and impaired STAT3 signaling in CD31-deficient mice. Am J Pathol 2005;166 (1):185–196.
35. Maas M, Stapleton M, Bergom C, et al. Endothelial cell PECAM-1 confers protection against endotoxic shock. Am J Physiol Heart Circ Physiol 2005;288(1):H159–H164.
36. Losy J, Niezgoda A, Wender M. Increased serum levels of soluble PECAM-1 in multiple sclerosis patients with brain gadolinium-enhancing lesions. J Neuroimmunol 1999;99 (2):169–172.
37. Niezgoda A, Losy J. Pecam-1 expression in patients with relapsing-remitting multiple sclerosis. Folia Morphol (Warsz) 2002;61(3):143–145.
38. Kuenz B, Lutterotti A, Khalil M, et al. Plasma levels of soluble adhesion molecules sPECAM-1, sP-selectin and sE-selectin are associated with relapsing-remitting disease course of multiple sclerosis. J Neuroimmunol 2005;167(1–2):143–149.
39. de Fougerolles AR, Stacker SA, Schwarting R, Springer TA. Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1. J Exp Med 1991;174(1): 253–267.
40. Sundd P, Gutierrez E, Koltsova EK, et al. 'Slings' enable neutrophil rolling at high shear. Nature 2012;488 (7411):399–403.
41. Lyck R, Enzmann G. The physiological roles of ICAM-1 and ICAM-2 in neutrophil migration into tissues. Curr Opin Hematol 2015;22(1):53–59.
42. Halai K, Whiteford J, Ma B, et al. ICAM-2 facilitates luminal interactions between neutrophils and endothelial cells in vivo. J Cell Sci 2014;127(Pt 3):620–629.
43. Finsterbusch M, Voisin MB, Beyrau M, et al. Neutrophils recruited by chemoattractants in vivo induce microvascular plasma protein leakage through secretion of TNF. J Exp Med 2014;211(7):1307–1314.
44. Massaro AR, De Pascalis D, Carnevale A, Carbone G. The neural cell adhesion molecule (NCAM) present in the cerebrospinal fluid of multiple sclerosis patients is unsialylated. Eur Rev Med Pharmacol Sci 2009;13(5): 397–399.
45. Ziliotto N, Zivadinov R, Jakimovski D, et al. Plasma levels of soluble NCAM in multiple sclerosis. J Neurol Sci 2019;396:36–41.
46. Rieckmann P, Altenhofen B, Riegel A, et al. Soluble adhesion molecules (sVCAM-1 and sICAM-1) in cerebrospinal fluid and serum correlate with MRI activity in multiple sclerosis. Ann Neurol 1997;41(3): 326–333.
47. Rieckmann P, Altenhofen B, Riegel A, et al. Correlation of soluble adhesion molecules in blood and cerebrospinal fluid with magnetic resonance imaging activity in patients with multiple sclerosis. Mult Scler 1998;4(3):178–182.

