Increased cerebrospinal fluid metalloproteinase-2 is associated with blood-brain barrier disruption in neuromyelitis optica

（視神経脊髄炎において髄液MMP-2は上昇し、血液脳関門の破綻に関与する）

千葉大学大学院医学薬学府
先端医学薬学専攻
（主任：桑原　聡教授）
内田　　智彦

Abstract

Objective: Astrocyte damage mediated by anti-aquaporin-4 antibodies plays a central role in the pathogenesis of neuromyelitis optica (NMO), which presumably requires preceding blood–brain barrier (BBB) disruption. Although it is well known that matrix metalloproteinases (MMPs) contribute to BBB disruption in multiple sclerosis (MS), the mechanisms responsible for the breakdown of the BBB in NMO are unclear.
To study whether the changes in cerebrospinal fluid (CSF) and serum levels of MMPs, tissue inhibitors of metalloproteinases (TIMPs) and cytokines are associated with BBB disruption in NMO.

Methods: CSF and serum samples were obtained from 29 patients with NMO (19 with NMO and 10 anti-aquaporin-4 antibody-positive NMO spectrum disorder), 29 with relapsing-remitting multiple sclerosis (MS), and 27 with other neurological disorders (17 with non-inflammatory and 10 with inflammatory neurological disorders). We measured the concentrations of 27 bioactive substances, including 9 MMPs, 4 TIMPs, and 14 cytokines, using a multiplex assay. We performed immunohistochemistry for MMP-2 and TIMP-1 expression on post-mortem brain tissues from patients with NMO.

Results: In CSF but not serum samples, MMP-2, TIMP-1, interleukin-6 (IL-6) levels, and the MMP-2/TIMP-2 ratio were significantly elevated in patients with NMO. In the NMO group, the CSF/serum albumin ratio (a measure of BBB breakdown) revealed the highest correlation with CSF MMP-2 levels, which correlated with CSF IL-6 levels. Immunohistochemistry revealed MMP-2- and TIMP-1-positive cells surrounding the vessels in NMO lesions.

Conclusions: Increased CSF MMP-2 levels, likely enhanced by IL-6 signaling, may disrupt BBB from outside of the vessels and may enable serum anti-AQP4- antibodies to migrate to CNS across BBB in patients with NMO.

Introduction

Neuromyelitis optica (NMO) is an inflammatory demyelinating disease of the central nervous system (CNS), which is characterized by optic neuritis and longitudinally extensive myelitis.[1, 2] NMO-IgG was discovered in the sera of NMO patients and is recognized as a specific biomarker of NMO.[3] It has been revealed that this antibody targets aquaporin-4 (AQP4), a water channel protein localized at astrocytic foot processes, and is a key molecule in the pathogenesis of NMO.[4] Mechanisms for the disruption of the blood–brain barrier (BBB) and invasion of the anti-AQP4 antibody in the CNS remain unknown, although the cerebrospinal fluid (CSF)/serum albumin ratio (Q albumin, Qalb), a measure of BBB breakdown, is elevated in NMO.[5]

Several studies have shown that inflammatory cytokines and matrix metalloproteinases (MMPs) induce BBB disruption.[6-8] The MMPs are a family of zinc-containing and Ca^{2+}-requiring endopeptidases, mediating the degradation and remodeling of extracellular matrix (ECM) components, including fibronectin, laminin, elastin, collagen IV, and proteoglycans, in pathological and physiological processes such as tissue remodeling and wound healing.[9] Laminin, fibronectin, and collagen IV are reported to be the major ECM components of brain capillaries;[10] hence, MMPs, including MMP-2, -3, and -9, which are capable of degrading collagen IV, can be involved in BBB disruption.[6]

The activity of MMPs is regulated by various factors, including MMP gene expression, others MMPs, and tissue inhibitors of metalloproteinases (TIMPs). Moreover, MMP gene expression is regulated by Th-17-related cytokines such as tumor necrosis (TNF)-α, interleukin (IL)-1β, IL-6, and IL-17.[6, 11-13] Therefore, MMPs, TIMPs, and Th-17-related cytokines play an important role in BBB function.

Additionally, these molecules are related to BBB disruption and the transmigration of inflammatory cells in MS.[14, 15]

In this study, we aimed to elucidate the role of MMPs in disruption of BBB, and to investigate the association of MMPs with TIMPs and Th-17-related cytokines in NMO.

Methods

Patients

Patients enrolled in this study were seen at the Chiba University Hospital between 2000 and 2014. Twenty-nine patients had relapsing-remitting MS (6 of which were men), fulfilling the 2010 revisions of the McDonald criteria,[16] and 29 patients had NMO, including 19 patients fulfilling the 2006 Wingerchuk NMO criteria[17] and 10 anti-AQP4 antibody-positive NMO spectrum disorder (NMOSD) patients fulfilling the 2015 Wingerchuk criteria.[18] Clinical relapses were defined as the outbreak of any new neurological symptoms or the worsening of previous symptoms associated with a clinical sign persisting longer than 24 h in the absence of fever, in patients who were previously clinically stable for at least 30 day. Twenty-seven patients had other neurological diseases (ONDs), including 17 with non-inflammatory neurological disorders (5 Parkinson's disease, 1 corticobasal degeneration,1 progressive supranuclear palsy, 3 multiple system atrophy, 1 spinocerebellar degeneration, and 6 amyotrophic lateral sclerosis) and 10 with inflammatory neurological disorders (4 Neuro-Behçet disease, 4 neurosarcoidosis, and 2 CNS lupus).

We reviewed the gender, age, disease duration, Krutzke's expanded disability status scale (EDSS)[19] score, presence of longitudinally extensive (≥3 vertebral segment) spinal cord lesions (LESCL), serum anti-AQP4 antibody positivity,[20]

oligoclonal IgG bands positivity, IgG index, whether the brain magnetic resonance imaging (MRI) fulfilled the Paty's MRI criteria,[21] positive gadolinium-enhancing brain and spinal cord lesions on MRI, and whether patients had received any immunomodulating therapy at the time of sample collection, such as interferon (INF)-β, fingolimod, continuous oral prednisolone, or azathioprine.

This study was approved by the Ethics Committee of the Chiba University School of Medicine. All the participants gave informed consent for their participation in this study.

CSF and Serum Sampling

CSF and serum samples were obtained from NMO and MS patients at clinical relapse, within 50 days of the relapse onset before treatment for relapse. All samples were stored at -80 ºC until analysis.

MMP, TIMP, and cytokine concentrations

The concentration of 9 MMPs (MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-13), 4 TIMPs (TIMP-1, TIMP2, TIMP-3 and TIMP-4), and 14 cytokines (IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN-γ, and TNF-α) in the CSF were measured using the Bio-Plex ProTM® magnetic bead-based multiplex immunoassay (Bio-Rad, Hercules, CA, USA). The fluorescence intensity from the immunoassay was acquired and analyzed using the Bio-PlexTM 200 System (software version 6.1; Bio-Rad Laboratories). MMPs, TIMPs, and cytokines in the CSF that were significantly different between NMO, MS, and OND patient groups were also measured in the sera of these three groups. For

statistical analysis, the concentrations below the lower limit of detection were replaced by a value equivalent to half the lower limit of detection.

CSF/serum ratio of elevated MMPs, TIMPs, and cytokines in the NMO patients

The CSF/serum ratio of elevated MMPs, TIMPs, and cytokines in NMO patients was calculated from the measured concentrations, if those levels were directly measured without replacement.

Magnetic resonance imaging (MRI)

MRI scans were taken exclusively at symptom onset and within 60 days of onset. A 1.5-T MRI scanner was used to perform brain and spinal cord MRI. Lesions showing gadolinium enhancement on T1-weighted images were defined as active lesions.

Correlation of CSF bioactive substances with Qalb or clinical, laboratory, and radiological findings in NMO patients

We first analyzed the correlations between CSF bioactive substances, including MMPs, TIMPs, and cytokines, and Qalb to investigate the association between BBB permeability and these substances in NMO patients. Secondly, we identified CSF bioactive substances that were significantly different between NMO and MS patients or between NMO and OND patients. We then analyzed the correlation between the identified bioactive substances and the clinical, laboratory, and radiological findings of the NMO patients, including the EDSS, CSF cell count, CSF protein concentration, IgG index, and MRI findings.

Immunohistochemical detection of MMP-2 and TIMP-1with NMO

We performed immunohistochemical analysis for AQP4, MMP-2, and TIMP-1 on post-mortem brain tissues obtained from two patients with NMO (69- and 44-year-old females with disease durations of 12 and 4 years, respectively). The primary antibodies used were the anti-AQP4 rabbit antibody (Sigma-Aldrich, dilution 1:100, USA), anti-MMP-2 mouse antibody (clone: 17B11, Leica Biosystems, dilution 1:200, Germany), and anti-TIMP-1 monoclonal antibody (clone: 6F6a, Leica Biosystems, dilution 1:1000, Germany).

Statistical analysis

The Mann–Whitney U test with the Holm adjustment and Fisher's exact probability test were conducted to compare groups. Spearman's rank correlation analysis was used to assess correlation. A *P* value below 0.05 was considered statistically significant. All statistical analyses were performed using SPSS statistical software, version 16.0J (SPSS Japan Inc, Tokyo, Japan).

Results

Clinical characteristics of the patients

The demographic and clinical characteristics, laboratory findings, MRI findings, and treatments of the patients with NMO and MS are shown in **Table 1**. The sex ratio, age, disease duration, CSF cell count, CSF protein concentration, IgG index, the rate of patients whose MRI fulfill the Paty's criteria,[20] and the rate of patients with gadolinium-enhancing lesions on MRI, were each similar between the NMO and MS groups. EDSS scores, positivity for anti-AQP4 antibodies, Qalb, negativity for

oligoclonal IgG bands, positivity for having LESCL on previous spinal cord MRI, and the frequency of patients with any immunomodulating therapy and continuous oral prednisolone therapy were higher in the NMO group than in the MS group.

CSF and serum MMP, TIMP, and cytokine profiles

CSF and serum MMP, TIMP, and cytokine profiles of NMO, MS, and ONDs are presented in **Table 2**. Of the MMPs, TIMPs, and cytokines, significant differences between any two of the three patient groups are shown in **Figure 1**. CSF MMP-2, TIMP-1, and IL-6 levels were significantly higher in NMO patients than in both MS and OND patients. CSF IL-17A levels were significantly higher in MS patients than in both NMO and OND patients, and CSF MMP-13 levels were significantly higher in MS patients than in OND patients (**Figure 1**). The CSF MMP-2/TIMP-2 ratio was significantly elevated in NMO patients compared with MS and OND patients. There were no significant differences in other CSF MMPs, TIMPs and cytokines among the three groups (**Table 2**). CSF MMPs, TIMPs and cytokines, significant differences between any two of the three groups, as well as MMP-9, were also measured in the sera of the three groups of patients; however, no significant differences in any of these factors were observed between any of the groups (**Table 2**).

CSF/serum ratios of MMP-2, TIMP-1, and IL-6 in NMO patients

The CSF/serum ratios of MMP-2, TIMP-1, and IL-6 in the NMO patient group are shown in **Figure 2**. The median CSF/serum ratios of MMP-2 and IL-6 were over 1.0, indicating these substances were predominantly produced in the CNS. The median ratio values for MMP-2, TIMP-1, and IL-6 were 1.25 (interquartile range [IQR]: 0.95),

0.21 (IQR: 0.17), and 1.47 (IQR: 1.12), respectively.

MRI findings

MRI scans performed at clinical onset were available for 23 of 29 NMO patients and for 21 of 29 MS patients. Active lesions with gadolinium enhancement on MRI were observed in 11 of the 23 NMO patients, and in 12 of the 21 MS patients, although the frequency was not significantly different between NMO and MS groups.

Correlation of CSF bioactive substances with Qalb and with MRI, clinical, laboratory, and radiological findings in NMO patients

Qalb was correlated with CSF levels of MMP-2, MMP-3, MMP-7, MMP-10, TIMP-1, IL-6, IL-10, and TNF-α in NMO patients (**Table 3**). CSF bioactive substances that were significantly different either between NMO and MS or between NMO and OND patients, and which were also significantly correlated with Qalb, included only MMP-2 ($\rho = 0.753$; $P < 0.0001$), TIMP-1 ($\rho = 0.604$; $P = 0.001$), and IL-6 ($\rho = 0.450$; $P = 0.021$) (**Figure 3A–C**). CSF MMP-2 showed the highest correlation with Qalb, we analyzed the association of MMP-2 with other bioactive substances and with clinical, laboratory, and radiological findings. CSF MMP-2 levels showed correlation with CSF TIMP-1 ($\rho = 0.669$; $P < 0.0001$), IL-6 levels ($\rho = 0.547$; $P = 0.002$) (**Figure 3D and E**), CSF proteins ($\rho = 0.751$; $P < 0.0001$), the IgG index ($\rho = 0.598$; $P = 0.001$), and CSF cell counts ($\rho = 0.493$; $P = 0.008$; **Figure 3F–H**), but not with other clinical, laboratory, or radiological findings. Furthermore, CSF IL-6 was correlated with TIMP-1 ($\rho = 0.691$; $P < 0.0001$)

Immunohistochemical analysis

In post-mortem brain tissues, MMP-2 (**B, E**) and TIMP-1 (**C, F**) weakly positive cells were suspected to be macrophages and were observed around the vessels in NMO lesions **(Figure 4)** where AQP4 was lost (**A**, **D**).

Discussion

Our results show that CSF levels of MMP-2, TIMP-1 and IL-6 were significantly elevated in NMO patients and that CSF MMP-2 levels are significantly correlated with Qalb and IL-6 levels in NMO patients. Taken together, our findings suggest that upregulated MMP-2 is associated with the breakdown of BBB.

Few studies have demonstrated the role of MMP on BBB disruption in NMO pathogenesis. Serum MMP-9 levels and CSF TIMP-1 levels were significantly elevated, and serum MMP-9 levels were significantly correlated with Qalb in patients with NMO. [22] Moreover, sera from patients with NMO significantly increased the autocrine MMP-2/9 secretion in cultured human brain microvascular endothelial cells and decreased BBB function, which could be reversed by a broad inhibitor of MMPs (including MMP-2 and MMP-9). [23] Although these findings suggest that MMPs are involved in BBB disruption in patients with NMO, CSF MMP-2 is not examined. In our study, CSF MMP-2 levels are elevated in CSF but not in the serum of patients with NMO and are strongly correlated with Qalb. Moreover, the CSF/serum MMP-2 ratio suggests that MMP-2 is predominantly produced in CNS. Immunocytochemistry revealed MMP-2 positive cells, probable macrophages, around the vessels. We suggest that elevated CSF MMP-2 levels reached the ECM composing BBB from

outside of the blood vessels to increase BBB permeability. Recent studies have shown that pericytes, the outermost component of BBB, express MMP-2, -3, and -9 receptors and play an important role in neurovascular function, including BBB formation.[24] Pieper et al. reported that pericytes support the transmigration of neutrophils, and a specific inhibitor of MMP-2 and MMP-9, which significantly decreases the effect of transmigration from outside the cerebral small vessel in NMO, may first act on pericytes.[25]

Several studies have demonstrated that a regulatory pathway for MMP and TIMP expressions is mediated by IL-6. Elevated IL-6 levels were significantly correlated with mRNA and protein levels of MMP-2/9 and TIMP-1. [11, 12, 26, 27] We observed similar findings in CSF in NMO patients. Therefore, CSF IL-6 may induce MMP-2 and TIMP-1 production in CNS.

The elevation of CSF MMP-2, TIMP-1, and IL-6 levels seen in NMO is not seen in MS. BBB disruption is important in the pathogeneses of NMO and MS, but the underlying mechanism of BBB disruption may be different between these two disorders. This difference may be caused by high CSF levels of IL-6, which could induce MMP-2 and TIMP-1 production in the CNS. Therefore, IL-6 could potentially be used as a biomarker to differentiate NMO from MS.

Furthermore, other factors could regulate MMP-2 activity. Plasmin can activate MMP-2, while plasmin inhibitors and anti-urokinase plasminogen activator antibodies can inhibit pro-MMP-2 activation. [28] Angiotensin II (ANG II) induced MMP-2 release and reduced TIMP-2 selection. Moreover, the ANG II receptor blocker (ARB) prevented these effects. [29] Hence, a plasmin inhibitor and ARB could be novel therapeutic agents in patients with NMO via MMP-2 suppression.

Our study has several limitations. First, we were unable to clarify which cells produce MMP-2, TIMP-1, and IL-6 and sufficiently define the order of production of these three bioactive substances. Further studies, perhaps using *in vitro* BBB models of cultured cells, to clarify which cells produce these bioactive substances, and in what order, are therefore required. Second, generally, activated MMPs are derived from the activation of pro-MMPs. Our assays detect not only the activated MMP form but also the inactivated pro-forms; thus, further analysis specifically of activated MMPs using zymography could be beneficial for the understanding of the association between BBB and MMPs.

In conclusion, our study suggests that CSF MMP-2 plays an important role in the disruption of BBB in NMO and that elevated CSF MMP-2 may be induced by IL-6 signaling. MMP-2 may be a novel therapeutic target for NMO.

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Conflict of Interest Disclosures: None reported.

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Figures&Tables

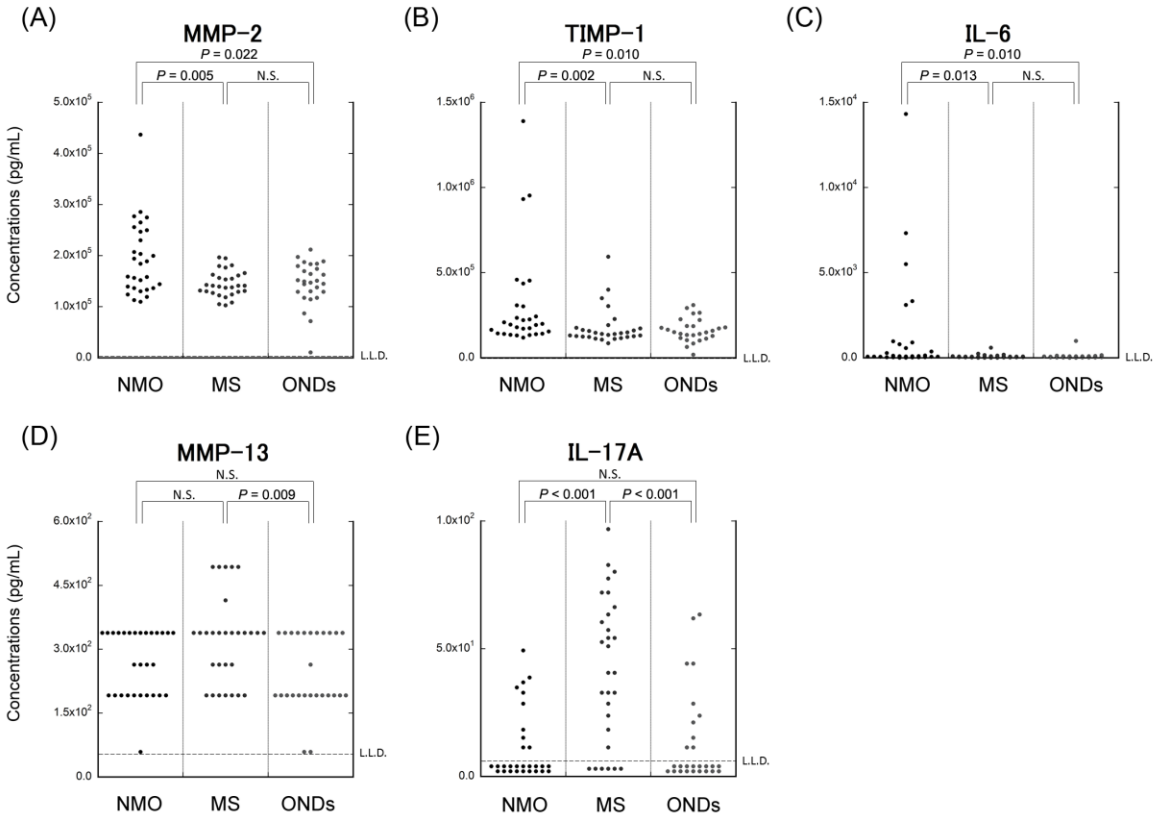

Figure 1 CSF MMP, TIMP, and cytokine concentrations in NMO, MS, and OND patients

Concentrations of matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinase (TIMPs), and cytokines in the cerebrospinal fluid (CSF) of 29 neuromyelitis optica (NMO) patients, 29 multiple sclerosis (MS) patients, and 27 patients with other neurological disorders (ONDs). Dashed lines indicate the lower limit of detection (L.L.D.).

*Statistically significant differences even after multiple comparison adjustment.

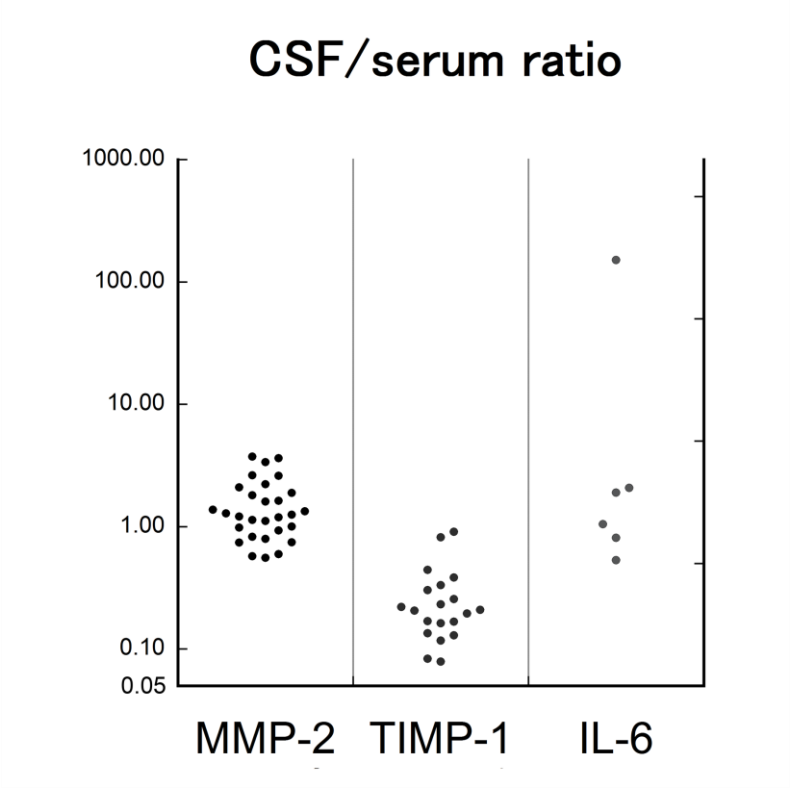

Figure 2

CSF/serum ratios for MMP-2, TIMP-1, and IL-6 in NMO patients

The median CSF/serum ratios of MMP-2 and IL-6 were over 1.0, which indicates they were dominantly produced in the central nervous system. The median ratio values for MMP-2, TIMP-1, and IL-6 were 1.25 (interquartile range [IQR]: 0.95), 0.21 (IQR: 0.17), and 1.47 (IQR: 1.12), respectively.

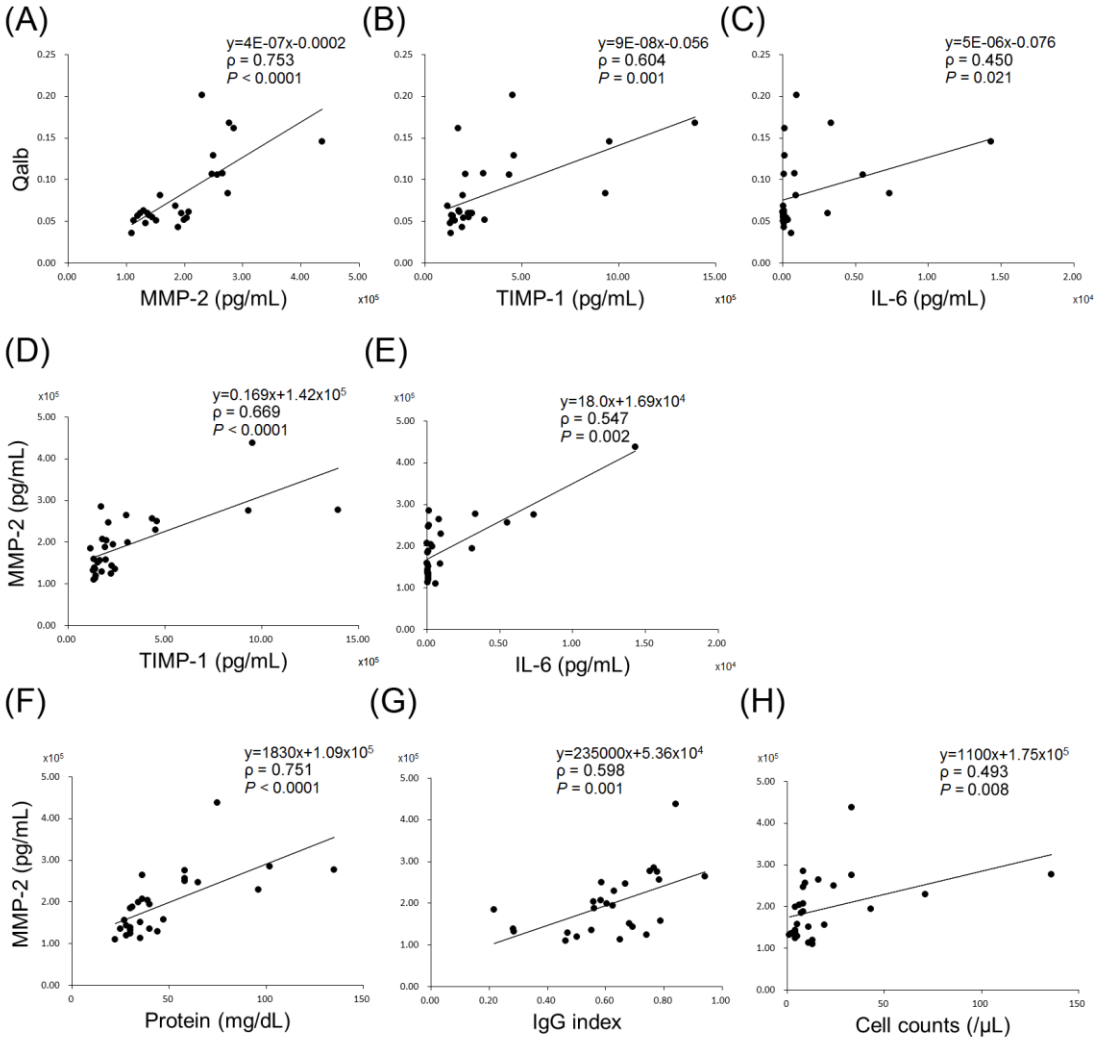

Figure 3 Correlations between CSF /serum albumin ratios (Qalb) and CSF bioactive substances, including MMPs, TIMPs, and cytokines

Correlations were tested between CSF MMP-2 and other CSF MMPs, TIMPs, and cytokines levels (D, E), and between CSF MMP-2 and other CSF parameters (F–H) in NMO patients. Only CSF levels of MMP-2, TIMP-1, and IL-6 were significantly correlated with Qalb (A–C). CSF MMP-2 levels showed significant correlation with CSF levels of TIMP-1, IL-6, CSF proteins, IgG index, and CSF cell counts (D–H).

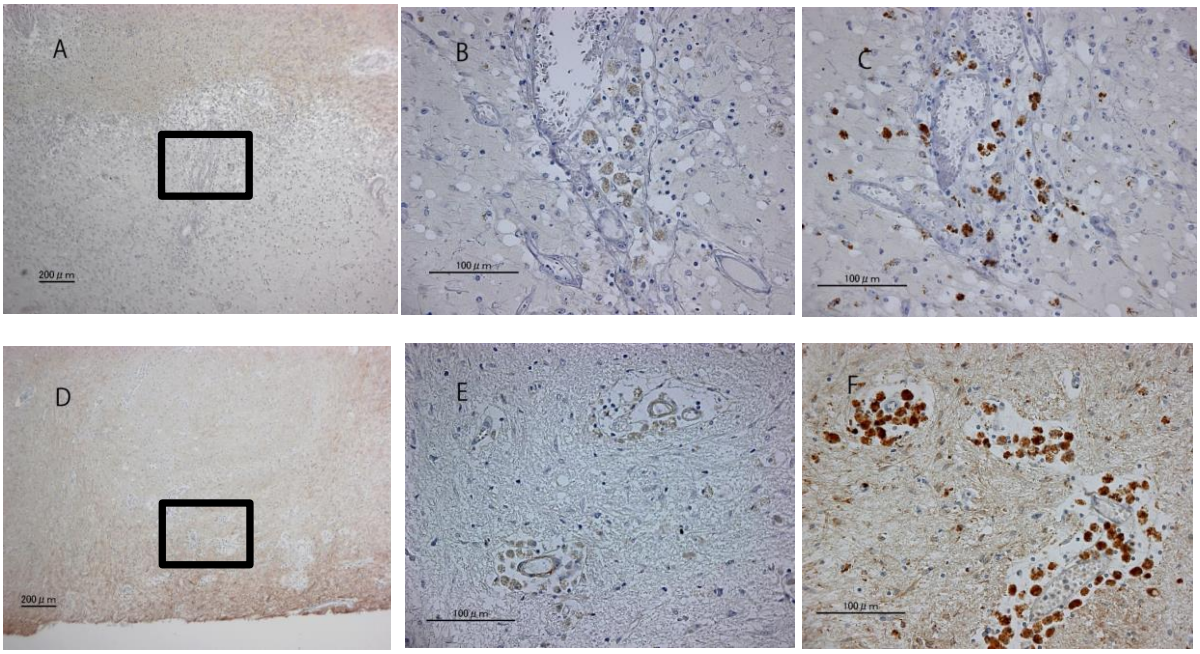

Figure 4 Immunohistochemical assessment of MMP-2 and TIMP-1 on post-mortem brain tissues from two NMO patients

A post-mortem neuropathological study was performed with two NMO patients (pt1 and pt2) using the brainstem (pt1 and pt2) tissues. We performed immunohistochemistry for AQP4 (A, D), MMP-2 (B, E), and TIMP-1 (C, F). MMP-2 weakly positive and TIMP-1-positive large cells, suspected to be macrophages, were detected around the vasculature in the NMO lesions where AQP4 was lost (A, D).

Table 1. Demographic and clinical characteristics, laboratory and MRI findings, and treatments in Patients with NMO and MS.

Clinical Characteristics	[a]NMO (*n* = 29)	MS (*n* = 29)	*P* value
Demographic and clinical characteristics			
Men:women	2:27	6:23	0.12
Age, year; median (IQR)	52 (20)	36 (12)	0.15
Disease duration, month; median (IQR)	65 (154)	49 (124)	0.31
EDSS; median (IQR)	6.0 (3)	2.5 (2)	0.001
Laboratory and MRI findings			
Positive aquaporin-4 antibody	24/29 (83%)	0/29 (0%)	<0.001
CSF cell count (IQR)	8 (12.0)	11 (23.5)	0.44
CSF protein concentration (IQR)	36 (28.0)	32 (8.5)	0.17
Qalb (IQR)	60.8 (51.9)	44.1 (13.3)	<0.001
IgG index (IQR)	0.6 (0.2)	0.7 (0.4)	0.06
Positive oligoclonal IgG bands	7/26 (27%)	16/27 (59%)	0.01
Fulfilling Paty's MRI criteria	19/29 (66%)	22/29 (76%)	0.28
≥3 vertebral segment spinal cord lesion	22/29 (76%)	2/29 (7%)	<0.001
Positive Gd-enhancing brain lesion	12/24 (50%)	12/21 (57%)	0.63
Treatments (Immunomodulating therapy)			
Any treatment	14/29 (48%)	2/29 (7%)	0.001
Interferon-β	1/29 (3%)	2/29 (7%)	0.55

Fingolimod	0/29 (0%)	0/29 (0%)	-
Continuous oral prednisolone	14/29 (48%)	0/29 (0%)	<0.001
Azathioprine	1/29 (3%)	0/29 (0%)	0.36

[a]CSF, cerebrospinal fluid; EDSS, Krutzke's expanded disability status scale; Gd, gadolinium; IQR, interquartile range;

MRI, magnetic resonance imaging; MS, multiple sclerosis; NMO, neuromyelitis optica spectrum; ONDs, other neurological disorders; Qalb, cerebrospinal fluid/serum quotients of albumin.

Table 2. CSF and serum MMP, TIMP, and cytokine profiles of NMO, MS, and OND patients

[b]MMPs/TIMPs/Cytokines (pg/mL)			NMO (*n* = 29)	MS (*n* = 29)	ONDs (*n* = 27)	P value		
						NMO vs. MS	NMO vs. ONDs	MS vs. ONDs
CSF	MMPs	MMP-1	[a]114 (236)	288 (174)	114 (236)	0.120	0.678	0.063
		MMP-2	184189 (110537)	140916 (31838)	149457 (47649)	**0.005***	**0.022***	0.451
		MMP-3	1096 (1263)	1096 (962)	442 (654)	0.730	0.115	0.119
		MMP-7	1242 (430)	1242 (571)	954 (509)	0.404	0.069	0.037
		MMP-8	2802 (897)	2605 (618)	2858 (578)	0.888	0.476	0.222
		MMP-9	508 (351)	686 (354)	508 (289)	0.943	0.021	0.031
		MMP-10	972 (507)	715 (257)	972 (323)	0.195	0.254	0.817
		MMP-12	22.2 (0.00)	22.2 (0.00)	22.2 (0.00)	0.161	0.492	1.000
		MMP-13	264 (147)	338 (75)	192 (147)	0.066	0.317	**0.009***
	TIMPs	TIMP-1	194900 (159162)	140276 (48062)	150459 (63917)	**0.002***	**0.010***	0.757
		TIMP-2	64201 (15523)	69254 (20849)	64793 (17531)	0.055	0.773	0.037
		TIMP-3	99.4 (0.00)	99.4 (0.00)	99.4 (0.00)	0.654	1.000	0.612
		TIMP-4	72.4 (0.00)	72.4 (0.00)	72.4 (0.00)	0.082	-	0.237

Table 2. CSF and serum MMP, TIMP, and cytokine profiles of NMO, MS, and OND patients

Cytokines	IL-1β	0.49 (0.00)	0.49 (0.00)	0.49 (0.00)	0.418	0.112	0.492
	IL-4	9.40 (0.00)	9.40 (0.00)	9.40 (0.00)	-	-	-
	IL-6	89.3 (737.7)	59.1 (43.4)	46.9 (52.8)	**0.013***	**0.010***	0.848
	IL-10	1.57 (31.52)	1.57 (11.03)	1.57 (11.03)	0.150	0.484	0.547
	IL-17A	2.96 (12.15)	40.58 (43.08)	2.96 (15.16)	**<0.001***	0.780	**<0.001***
	IL-17F	18.33 (0.00)	18.33 (0.00)	18.33 (0.00)	0.326	1.000	0.482
	IL-21	142 (0.00)	141.70 (0.00)	141.70 (0.00)	-	-	-
	IL-22	9.37 (0.00)	9.37 (0.00)	9.37 (0.00)	0.421	0.503	0.858
	IL-23	13.0 (13.8)	13.0 (43.1)	13.0 (0.00)	0.668	0.328	0.196
	IL-25	2.18 (0.00)	2.18 (0.00)	2.18 (0.00)	0.169	1.000	0.286
	IL-31	23.5 (0.00)	23.5 (0.00)	23.5 (0.00)	-	-	-
	IL-33	93.4 (29.7)	89.7 (29.7)	97.0 (18.4)	0.981	0.655	0.504
	IFN-γ	15.7 (0.00)	15.7 (0.00)	15.7 (0.00)	0.977	0.479	0.598
	TNF-α	9.28 (5.64)	6.83 (3.39)	7.66 (4.96)	0.068	0.389	0.436
	MMP-2/TIMP-2	2.77 (1.56)	2.00 (0.66)	2.28 (0.62)	**<0.001***	**0.01***	**0.016***

Table 2. CSF and serum MMP, TIMP, and cytokine profiles of NMO, MS, and OND patients

		MMP-9/TIMP-1	0.0031 (0.0028)	0.0038 (0.0035)	0.0032 (0.0032)	0.084	0.745	0.221
Serum	MMPs	MMP-2	127110 (49016)	123155 (44039)	136748 (65846)	0.243	0.742	0.195
		MMP-9	104453 (138410)	105913 (156113)	101562 (179452)	0.731	0.880	0.883
		MMP-13	86.1 (82.5)	44.5 (118.7)	44.5 (77.7)	0.187	0.219	0.927
	TIMPs	TIMP-1	577313 (974497)	43203 (467722)	400274 (1015322)	0.061	0.711	0.177
	Cytokines	IL-6	2.32 (0.00)	2.32 (0.00)	2.32 (82.51)	0.892	0.266	0.321
		IL-17A	3.22 (0.00)	3.22 (0.00)	3.22 (0.00)	0.082	0.799	0.228

[a]Data are presented as median (interquartile range within parentheses).

[b]CSF, cerebrospinal fluid; IFN, interferon; IL, interleukin; MMP, matrix metalloproteinase; MS, multiple sclerosis; NMO, neuromyelitis optica; ONDs, other neurological disorders; TIMP, tissue inhibitors of metalloproteinases; TNF, tumor necrosis factor.

* Statistically significant after multiple comparison adjustment.

Table 3. Correlation between Qalb and levels of CSF MMPs, TIMPs, and cytokines in NMO patients.

[a]CSF MMPs	Spearman's rank correlation coefficient (ρ)	*P* value	CSF cytokines	Spearman's rank correlation coefficient (ρ)	*P* value
MMP-1	−0.446	0.022	IL-1β	0.382	0.054
MMP-2	0.753	<0.0001	IL-4	N.A.	N.A.
MMP-3	0.407	0.039	IL-6	0.450	0.021
MMP-7	0.580	0.002	IL-10	0.529	0.006
MMP-8	−0.267	0.188	IL-17A	0.157	0.444
MMP-9	0.087	0.673	IL-17F	0.253	0.212
MMP-10	0.531	0.005	IL-21	N.A.	N.A.
MMP-12	−0.023	0.912	IL-22	0.157	0.443
MMP-13	−0.051	0.806	IL-23	−0.229	0.260
TIMP-1	0.604	0.001	IL-25	0.199	0.330
TIMP-2	0.243	0.232	IL-31	N.A.	N.A.
TIMP-3	0.000	1.000	IL-33	-0.142	0.491
TIMP-4	N.A.	N.A.	IFN-γ	-0.155	0.451
			TNF-α	0.552	0.004

[a]CSF, cerebrospinal fluid; IFN, interferon; IL, interleukin; MMP, matrix metalloproteinase; N.A., not available; NMO, neuromyelitis optica; TIMP, tissue inhibitors of metalloproteinases; TNF, tumor necrosis factor; Qalb, cerebrospinal fluid/serum quotients of albumin.

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