学位論文

「The Pathogenic Role of Lupus-Specific Autoantibodies and Interleukin-6 on Demyelination of the Brainstem and Spinal Cord in Systemic Lupus Erythematosus」

（全身性エリテマトーデスにおける脳幹脊髄の脱髄性症候群に対するループス特異的自己抗体とインターロイキン-6の病態解明）

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著者の宣言

本学位論文は、著者の責任において実験を遂行し、得られた真実の結果に基づいて正確に作成したものに相違ないことをここに宣言する。

論文要旨：

【背景】

全身性エリテマトーデス（SLE）は様々な自己抗体の産生を特徴とする慢性炎症性自己免疫疾患である。SLE に起因する神経障害は神経精神ループス（neuropsychiatric SLE: NPSLE）と呼ばれ、脳や脊髄、末梢神経における実質的な障害だけでなく、精神症状を伴う中枢神経系（CNS）の機能障害を含みその病型は多岐にわたる。NPSLE による中枢神経障害は SLE における生命予後を規定する主要臓器障害と位置づけられている。また中枢神経病変は全身性の疾患活動性と必ずしも相関せず、診断や疾患活動性をするための特異的バイオマーカーは未だ完全には確立していない。

しかしながら、SLE に合併した精神症状の発症メカニズムは明らかになりつつある。脳血液関門（BBB）の破綻により神経細胞に作用する自己抗体が CNS 内に流入し、神経細胞障害を来すことがループス精神病モデルマウスを用いた実験で示された。このことから、BBB の破壊と自己抗体の CNS 流入、神経細胞の障害というメカニズムが NPSLE 発症に関与している。

近年、免疫介在性の中枢神経疾患として視神経脊髄炎スペクトラム障害（NMOSD）が多発性硬化症とは独立する脱髄性疾患の疾患概念として確立した。その理由として NMOSD では疾患特異的自己抗体が発見されたこと、これに基づき従来の脱髄性疾患に行われてきた免疫賦活化ではなく免疫抑制療法が NMOSD では明らかに有用だったからである。これらは自己抗体の存在と疾患の発症が密接に関係したメカニズムがあることを明白に示唆している。NMOSD 疾患特異的自己抗体である抗アクアポリン 4（AQP4）抗体はアストロサイトに直接結合し補体活性化を開始し神経細胞に脱髄を引き起こす「機能」を持つ自己抗体であることが証明されている。すなわち自己抗体の存在自体が疾患の診断と発症メカニズムに直接関与しているということである。NMOSD の発症にもループス精神病におけるメカニズムと同様に抗 AQP4 抗体の BBB を越えた CNS への流入が必須と考えられている。興味深いことに NMOSD においては BBB の破綻を引き起こす抗グルコース制御タンパク質 78（GRP78）抗体の存在も明らかになっている。このように NMOSD で証明された一連の障害は、NPSLE にも共通する病態である。

一方、NPSLE に合併した脱髄では NMOSD に比べ抗 AQP4 抗体陽性率は低いことが報告されている。このことから共通するメカニズムがある一方、関与する自己抗体については差違があると考えられる。自己抗体以外のバイオマーカーとしてサイトカインの関与が考えられている。そのうち脳脊髄液（CSF）中のインターロイキン（IL）-6 濃度は NMOSD 疾患活動性と相関関係があることが報告されている。これまでわれわれはびまん性 NPSLE における CSF IL-6 の診断的有用性について報告してきた。そのため CSF IL-6 は画像的、メカニズム的に NMOSD の類似性の高い脱髄性の脳幹脊髄病変を有する NPSLE 患者のバイオマーカーとしても有用であると考えた。これら中枢神経病変に特化した NMOSD の研究にて得られた知見を元に、全身疾患の 1 つとして中枢神経病変を生じる NPSLE におけるメカニズムの解明をおこない診断的マーカー、疾患活動生マーカーが確立することで今

後の NPSLE を呈する SLE 患者診療の最適化の一助となると考えられる。

【目的】

脱髄性脳幹脊髄病変を有する NPSLE 患者において SLE 特異的自己抗体を含む免疫学的指標および IL-6 と中枢神経病変評価指標である脳脊髄液所見および画像的所見との関係性を明らかにし、SLE における NMOSD 様の病変と関連するバイオマーカーの特定よびメカニズムの解明に迫ることである。

【方法】

北里大学病院で 2005 年から 2019 年に脳幹脊髄に脱髄性病変を呈した SLE 患者の臨床データを後方視的に収集した。対照として同期間において MRI に異常所見のないびまん性 NPSLE 患者（びまん性 NPSLE 群）を選定した。SLE 診断は 1997 年米国リウマチ学会 SLE 分類基準を満たすものと定義した。視神経炎、脳幹脊髄病変は 2015 年度 NMOSD 国際分類基準で定義された MRI 画像所見(視神経炎や 3 椎骨を超える縦に長い横断性脊髄炎（LETM)、脳幹脳炎)を元に分類した。サブグループとして NMOSD 国際分類基準の定義にかかわらず、視神経炎や脳幹・脊髄の脱髄病変を有する SLE 患者を NMOSD 様 CNS 病変群とした。また同群の患者を抗 AQP4 抗体の有無を問わず NMOSD 国際分類基準の画像定義を完全に満たす患者（典型的 NMOSD 群）と、画像定義を完全に満たさないが脳幹脊髄の脱髄病変を有する患者（NMOSD 様脱髄群）に分類した。抗リン脂質症候群による血栓塞栓症の患者は除外した。すべての MRI 画像は当院の放射線科医により読影された。BBB 透過性は CSF/血清アルブミン比（QAlb：CSF/血清アルブミン×1,000）を用いて評価した。

【結果】

全 NPSLE 患者 80 人のうち、NMOSD 様 CNS 病変群、びまん性 NPSLE 群共に 12 人が抽出された。NMOSD 様 CNS 病変群のサブグループうち典型的 NMOSD 群は 8 人、NMOSD 様脱髄群 4 人であった。データが得られた患者で NMOSD 様 CNS 病変群の治療前 CSF IL-6 は 29.1 pg/mL (中央値)であり、治療後に 3.8 pg/mL へ有意に低下した（n=8, p=0.008)。典型的 NMOSD 群では CSF IL-6 は 8.3 pg/mL から.5.9 pg/mL へ低下し（n=5, p=0.063)、NMOSD 様脱髄群では 34.1 pg/mL から 2.0 pg/mL (n=3, 統計解析せず)へ低下した。NMOSD 様 CNS 病変群のうち 3 人死亡した。生存患者では全例で治療後 MRI 所見の改善を認めた。NMOSD 様 CNS 病変群において抗 AQP4 抗体陽性率は 18.2%だった。NMOSD 様 CNS 病変群において QAlb を測定された 9 人中 6 人（66.7%）において QAlb が 9 以上と BBB 破綻をきたしていた。治療前の CSF IL-6 値と自己抗体の関連については NMOSD 様 CNS 病変群では治療前の抗 dsDNA 抗体価と有意な相関を認めた（r=0.676, p=0.027)。CSF IL-6 値と抗 Sm 抗体価の間には明らかな相関関係を認めなかったが（r=0.391, p=0.236)。一方、サブグループ別に解析を行うと典型的 NMOSD 群では CSF IL-6 と抗 Sm 抗体の間に有意な相関関係を認めた（r=0.847, p=0.025)。さらに治療前の QA l b と自己抗体の関連性については抗 Sm 抗体と QAlb においてそれぞれ NMOSD 様 CNS 病変群(r=0.807, p=0.014)、典型的 NMOSD 群(r=0.975, p=0.033)と有意な相関関係

が認められた。

【結論】

脳脊髄液中の IL-6 は SLE 患者の脳幹脊髄病変の疾患活動性の有用なサロゲートマーカーとなり得る。加えて NMOSD と同様に脱髄性脳幹脊髄病変を呈した SLE 患者の BBB の破綻に自己抗体が寄与していた。

目次

Introduction

Systemic lupus erythematosus (SLE) is a chronic and inflammatory autoimmune disease, characterized by the production of various autoantibodies.[1] Nervous system complications primarily associated with SLE include neuropsychiatric syndromes in SLE (NPSLE). Various neurological disorders, including parenchymal damages in the brain, spinal cord, and peripheral nerves, and functional central nervous system (CNS) disorders presenting with psychiatric manifestations could occur.[2] However, no diagnostic criteria or biomarkers for disease activity have been established.

Recently, neuromyelitis optica spectrum disorder (NMOSD) has been defined as an autoimmune CNS demyelinating disease, characterized by the development of severe optic neuritis and transverse myelitis with the presence of antiaquaporin 4 (anti-AQP4) antibody (Ab).[3–5] For anti-AQP4 Ab to directly damage astrocytes, the blood–brain barrier (BBB) should be breached so that immunoglobulins can enter the CNS from systemic circulation beyond the BBB. As one of the mechanisms of BBB breaches, the autoantibody antiglucose-regulated protein 78 (GRP78) Ab was found in patients with NMOSD.[6]

Transverse myelitis and severe bilateral optic neuritis, which can lead to blindness, are distinct manifestations of NMOSD. Longitudinally extensive transverse myelitis lesions (LETMs) exceeding three vertebrae are the characteristic CNS findings in NMOSD. LETMs are also occasionally observed in patients with other autoimmune diseases.[3,7] Among NPSLE, demyelinating syndromes can rarely develop.[8] Besides, demyelination in patients with NPSLE can be involved in the development of brainstem and/or spinal cord lesions; particularly, spinal cord lesions often present with LETMs, some of which are similar to those observed in NMOSD.[9,10] In contrast, some cases of myelitis are not the same as those seen in LETMs,[10] indicating that demyelinating syndromes can present with various magnetic

resonance imaging (MRI) abnormalities, including those in NMOSD. Although anti-AQP4 Ab positivity is very low in NPSLE compared with that in NMOSD, the pathophysiology that depends on anti-AQP4 Ab is considered different; however, another autoantibody may contribute to the development of NMOSD-like CNS demyelinating lesions in autoantibody-producing autoimmune diseases, such as SLE.[11]

Among the biomarkers for NMOSD, cerebrospinal fluid (CSF) interleukin (IL)-6 has been reported to be closely related to disease activity.[12–14] Thus, we hypothesize that IL-6 is a useful biomarker for patients with NPSLE with brainstem and/or spinal cord lesions. In this study, we investigated the relationship of CSF IL-6 levels with the clinical characteristics of patients with NPSLE with brainstem and/or spinal cord lesions. Additionally, we investigated whether SLE-specific autoantibodies are associated with BBB breaches in patients with NPSLE with brainstem and/or spinal cord lesions because this could be one of the main mechanisms of autoantibody influx into the brain in NMOSD.

Methods

Patients

Patients with SLE admitted to Kitasato University Hospital for the treatment of NPSLE were recruited. The diagnosis of SLE was performed using the classification criteria for SLE established by the American College of Rheumatology and revised in 1997.[15] Data from patients who had primary NPSLE[2] with brainstem and/or spinal cord lesions admitted to Kitasato University Hospital from 2005 to 2019 were retrospectively collected and analyzed. As a control of demyelinating syndrome in brainstem and/or spinal cord lesions, data from

patients with diffuse psychiatric/neuropsychological manifestations of NPSLE[2] without MRI findings were also collected during the same analysis period.

Evaluation of CNS lesions

Optic neuritis, brainstem lesions, and LETMs were determined based on the patterns found in MRI scans as defined in the NMOSD international criteria: 1) optic neuritis or lesions involving more than half of the optic nerve with high T2 or T1 intensity by gadolinium enhancement, 2) LETMs or extensive spinal cord lesions consecutively encompassing more than three vertebrae with high T2 intensity, 3) brainstem lesions in the posterior medulla oblongata and area postrema, and 4) brainstem lesions mainly surrounding the ependyma.[3] Regardless of the imaging definition of the NMOSD international criteria, patients with optic neuritis or demyelination in brainstem or spinal cord lesions were defined as the NMOSD-like CNS lesions group, and patients with diffuse neuropsychiatric/neuropsychological NPSLE (diffuse NPSLE) without MRI abnormalities (diffuse NPSLE group) were included as a reference for autoantibody-based inflammatory CNS complications in SLE. For further analysis, patients in the NMOSD-like CNS lesions group were divided into two subgroups independent of the presence of anti-AQP4 Ab: those who completely met the image definitions from the NMOSD international criteria (atypical NMOSD) and those who did not completely meet the image definition but had demyelinating lesions (NMOSD-like demyelination). All MRI scans were analyzed by a radiologist at our hospital. Patients with thromboembolism due to antiphospholipid syndrome were excluded.

Clinical data acquisition

Patient background, clinical and serological data, and MRI findings were retrospectively collected from the medical charts. Disease activity was evaluated using the SLE Disease Activity Index (SLEDAI)-2K.[16] Antidouble-stranded deoxyribonucleic acid (anti-dsDNA),

anti-Smith (Sm), and antiribonucleoprotein Ab were measured using chemiluminescent enzyme immunoassay, anticardiolipin (CL) and anti-CL β2-glycoprotein were measured using enzyme-linked immunosorbent assay (ELISA), and lupus anticoagulant was measured using dilute Russell viper venom time. Anti-AQP4 Ab was measured using a commercially available ELISA or cell-based assay (CBA) (kindness of Dr. Takahashi, Department of Neurology, Tohoku University School of Medicine). Antimyelin-oligodendrocyte glycoprotein (MOG) Ab was measured as previously described.[11] Antiribosomal P protein (anti-ribo P) and anti-N-methyl-D-aspartate receptor subunit GluN2A/B (anti-GluN2) Ab were measured using our original in-house method based on previously described methods.[17,18] IL-6 was measured using a high-sensitivity ELISA (SRL, Inc., Tokyo, Japan). Baseline was defined as when the patient was admitted for treatment of NPSLE. Posttreatment evaluation was performed approximately 32.5 months (median) after the initial induction therapy (Table 1). BBB permeability was evaluated using CSF/serum albumin ratio (QAlb: CSF/serum albumin quotient × 1,000) as previously described.[19,20]

Statistical analysis

Fisher's exact test and the Mann–Whitney U-test were used to statistically analyze the comparisons between the patient groups. The interval changes before and after treatment were evaluated using Wilcoxon's signed-rank test. Correlations between CSF IL-6, QAlb, and serum Ab were calculated using Spearman's rank correlation coefficient. All data were analyzed using Prism 7 (GraphPad Software, Inc., San Diego, California, USA).

Informed consent

The Ethics Committee of Kitasato University Hospital (approval number: B17-153) approved this study. All patients provided written informed consent.

Results

Patient background and treatment

During the observation period, 80 patients with NPSLE were admitted, and 12 patients complicated with CNS demyelinating lesions in the brainstem and/or spinal cord (NMOSD-like CNS lesions group) and 12 patients with diffuse NPSLE were observed (Tables 1 and 2). Significant differences in the disease and follow-up durations were observed between the NMOSD-like CNS lesions and diffuse NPSLE groups. At baseline, the median SLEDAI-2K scores in the NMOSD-like CNS lesions and diffuse NPSLE groups were 13.0 and 19.5, respectively (Table 1). Of the 12 patients with NMOSD-like CNS lesions, eight had atypical NMOSD and four had NMOSD-like demyelination (Tables 3 and 4). No significant differences in background characteristics were observed between the atypical NMOSD and NMOSD-like demyelination subgroups. Two patients (Table 5) with NMOSD-like CNS lesions were the relapsed cases of NPSLE. A single case of sudden death right after the disease onset was observed in the NMOSD-like CNS lesions group. Eleven patients, except for the sudden death case, were treated with high-dose prednisolone, of whom 10 (90.9%) received steroid pulse therapy. In the NMOSD-like CNS lesions group, four patients (36.3%) received intravenous cyclophosphamide infusion therapy, two patients (18.2%) received high-dose intravenous immunoglobulin infusion, one patient (9.1%) complicated with lupus nephritis received mycophenolate mofetil, and one patient with macrophage activation syndrome (MAS) received cyclosporin A as an additional immunosuppressive therapy. In the atypical NMOSD subgroup, three patients who previously experienced NPSLE were observed only among whom two patients had demyelination recurrence, and other patients had NPSLE without demyelination. A fatal prognosis was observed only in patients with atypical

NMOSD. Overall, remission induction treatment successfully decreased SLEDAI-2K scores to 0 (range, 0–4) in patients with NMOSD-like CNS lesions (data not shown).

Brainstem and spinal cord lesions

1. Neurological findings

Subacute progression of neurological findings was observed in all patients within several days to weeks after the initial symptom. In the NMOSD-like CNS lesions group, sensory disorder was observed in nine patients (75.0%); muscular weakness was seen in seven patients (58.3%), characterized by paraplegia of the lower limbs; bladder and rectal disturbance was observed in four patients (25.0%); hiccups, vertigo, and double vision were seen in two patients (16.7%); and spasticity, nausea, and dysarthria were observed in one patient (8.3%). Sensory and motor disorders corresponded to the abnormal MRI findings. The neurological findings in the diffuse NPSLE group included delirium, psychosis, seizure, mood disorder and cognitive dysfunction. Although some patients remained paralyzed, improvement in neurological findings were observed following treatment (Table 5). One patient with atypical NMOSD had a cerebral hemorrhage, which was considered to be associated with thrombocytopenia caused by MAS.

2. MRI findings

In the NMOSD-like CNS lesions group, the most prevalent MRI findings were thoracic spinal cord lesions in six patients (50.0%), followed by cervical lesions in five patients (41.7%) and a lumbar lesion in one patient (8.3%). Five patients (41.7%) had brainstem lesions; two patients had lesions in the medulla oblongata, two patients had lesions in the pons, and one patient had a lesion in the midbrain. Besides, cerebral lesions were found in only two patients. Of the nine patients who underwent contrast-enhanced MRI, six (66.7%) had certain lesions with enhancements, among whom four patients (44.4%) showed ring enhancement around the

lesion and two patients (22.2%) had faint enhancement within the lesion. Overall, no difference in the MRI findings was observed between the atypical NMOSD and NMOSD-like demyelination groups, except for the size of the lesions, where the MRI findings were consistent with the NMOSD classification criteria. These MRI findings improved following treatment in cases that survived (Figure 1).

Serum autoantibodies and IL-6

Anti-AQP4 Ab, the specific autoantibody for NMOSD, was measured in the serum samples obtained from 11 patients with NMOSD-like CNS lesions (Table 2). Two patients (18.2%) who had positive results in CBA had negative results in ELISA. Anti-AQP4 Ab was not detected with either method in patients with NMOSD-like demyelination. Anti-MOG Ab was also not detected in any patient in both groups. Other specific autoantibody profiles were similar among both the groups (Table 2). Before treatment, the median serum IL-6 levels were 4.6 pg/mL in patients with NMOSD-like CNS lesions and 3.8 pg/mL in patients with diffuse NPSLE (Table 2). Unfortunately, serum IL-6 could not be measured after treatment due to lack of specimens.

CSF IL-6 interval changes

CSF was examined in 11 patients with NMOSD-like CNS lesions and in 12 patients with diffuse NPSLE (Table 2). The median cell number was 3 cells/μL (range, 0–154 cells/μL) and 0 cells/μL (range, 0–45 cells/μL), respectively. Protein levels were 77.0 mg/mL (range, 24–980 mg/mL) and 41.5 mg/mL (range, 26–152 mg/mL), respectively. QAlb was 11.5 (range, 4.1–116.4) and 30.6 (range, 5.6–33.2) in patients with NMOSD-like CNS lesions and those with diffuse NPSLE, respectively. Six of 9 patients (66.7%) in the NMOSD-like CNS lesions group whose QAlb was measured had QAlb over 9, indicating BBB disruption. In the NMOSD-like CNS lesions group, four patients were positive for myelin basic protein, three

patients had IgG index elevation, and three patients were positive for oligoclonal bands. The laboratory findings of patients with diffuse NPSLE are shown in Table 2.

Before treatment, the median CSF IL-6 was 29.1 pg/mL in patients with NMOSD-like CNS lesions and 10.0 pg/mL in patients with diffuse NPSLE. Posttreatment tests were performed in eight patients (five patients with atypical NMOSD and three patients with NMOSD-like demyelination) with a significant decrease in CSF IL-6 to 3.8 pg/mL ($p=0.008$) (Figure 2(a)). Five patients with atypical NMOSD and three patients with NMOSD-like demyelination underwent follow-up tests. CSF IL-6 was also decreased in both the atypical NMOSD and NMOSD-like demyelination groups; however, this decrease was not statistically significant (Figure 3(a) and 3(b)). In patients with diffuse NPSLE, the median CSF IL-6 levels also significantly decreased from 10.0 pg/mL to 3.2 pg/mL ($p=0.001$) (Figure 2(b)). No correlation was observed between CSF IL-6 and the risk of fatal prognosis or recurrence.

Relationship of serum autoantibodies with BBB integrity

Finally, we investigated the relationship between serum autoantibodies with serum IL-6 and CSF IL-6 levels before treatment. We found no statistical correlation between serum IL-6 and anti-dsDNA Ab, anti-Sm Ab, or CSF IL-6 in patients with NMOSD-like CNS lesions (Figure 4(a)–(c)). Although no statistically significant differences were found, QAlb tended to correlate with serum IL-6 (Figure 4(d)). In contrast, before treatment, CSF IL-6 showed a significant correlation with the anti-dsDNA Ab titer ($p=0.027$) (Figure 5(a)), but not with anti-Sm Ab ($p=0.236$) (Figure 5(b)). Of note, a significant correlation was observed between QAlb and the serum anti-Sm Ab titer ($p=0.014$) (Figure 5(d)) in patients with NMOSD-like CNS lesions ($p=0.027$).

Additionally, before treatment, a significant correlation between the serum anti-Sm Ab titer and CSF IL-6 ($p=0.025$) (Figure 6(b)) and QAlb ($p=0.033$) (Figure 6(d)) was observed in

patients with atypical NMOSD only. Meanwhile, QAlb significantly correlated with CSF IL-6 levels in patients with atypical NMOSD ($p=0.017$) (Figure 6(e)) before treatment. No statistical correlation was found between serum IL-6 and anti-dsDNA Ab, anti-Sm Ab, QAlb, or CSF IL-6 in patients with atypical NMOSD (Figure 7). Furthermore, no correlation was observed between other autoantibodies: antiphospholipid Ab, anti-ribo P Ab, anti-GluN2 Ab, and anti-AQP4 Ab (data not shown).

Discussion

This study demonstrated that CSF IL-6 levels significantly decreased following treatment, indicating improvements in neurological and MRI findings. Before treatment, CSF IL-6 was similar to that in patients with NMOSD, as described in previous reports.[12,13,21] These results suggest that even patients with SLE could have brainstem and/or spinal cord lesions that involve CSF IL-6 elevation, which is a common mechanism in NMOSD. Irrespective to the range of involved lesions observed by MRI, some of our patients with spinal cord demyelination resulted in elevated CSF IL-6 compared to previous reports on demyelination including MS and NMOSD. In addition, we have previously reported an autopsy case with SLE demonstrating demyelination complicated with vasculitis in the spinal cord.[22] Although IL-6 elevation does not reflect the specific inflammation process, unusual elevation of CSF IL-6 could be indicating the copresence of vasculitis in SLE patients with demyelination. However, further investigation with imaging and pathological analysis is needed to demonstrate these speculation.[22-24]

CSF IL-6 decreased following treatment in our patients with dNPSLE. Elevation of CSF IL-6 has been reported in dNPSLE[25] with other studies suggesting the activation of NF-κB pathway in activated endothelial cells by anti-NR2 Ab resulting in IL-6 production in the

CNS of NPSLE patients.[26] Our study demonstrated that the IL-6 production in CNS may be enhanced in demyelinating syndrome, not only in dNPSLE but also focal NPSLE. Regarding demyelination in SLE, we should consider applying immunosuppressive therapy, considering tissue damage, including astrocytes and neurons, manifested as demyelination and localized as vasculitis, when brainstem and/or spinal cord lesions with increased CSF IL-6 are found in patients with SLE as long as other causes cannot be present.

For the definitive diagnosis of NMOSD, the detection of anti-AQP4 Ab in the blood is particularly useful because of its high specificity. As a mechanism of the development of NMOSD, anti-AQP4 Ab binds directly to astrocytes because demyelination can be induced by complement activation after anti-AQP4 Ab binding to astrocytes.[5] For this reason, anti-AQP4 Ab in the blood must penetrate the BBB and reach the CNS to induce demyelination as astrocytopathy. A recent study has demonstrated that anti-GRP78 Ab, a monoclonal Ab derived from patients with NMOSD, can breach the BBB by binding to the surface of endothelial cells.[27] Taken together, in the pathogenesis of NMOSD, there is a two-step impairment caused by autoantibodies, resulting in demyelination after the breach of the BBB integrity. A similar mechanism can be involved in brainstem and/or spinal cord lesions in patients with SLE because SLE is also characterized by the presence of various autoantibodies like NMOSD.[28] We demonstrated that anti-GRP78 can be found in patients with SLE, which was significantly elevated in patients with NPSLE.[29] Besides, QAlb, the permeability index of the BBB, is higher in patients with lupus psychosis, which can be developed by the direct action of antineuron Ab, such as anti-GluN2 Ab.[30] Additionally, we demonstrated that QAlb in patients with lupus psychosis is significantly correlated with serum anti-Sm Ab,[19] suggesting that anti-Sm Ab can breach the BBB like anti-GRP78. In patients with SLE and NMOSD, anti-Sm Ab was correlated with CSF IL-6 and QAlb, which could be

related to a more permeable BBB, leading to severe demyelination caused by autoantibodies from the systemic circulation. We speculate that lupus psychosis and NMOSD-like CNS lesions have a common mechanism[31,32] and that NMOSD is caused by autoantibodies. Increased age and male sex are independently associated with the higher frequency of QAlb.[33,34] However, all of the patients were women under 60 years old in this study and had QAlb greater than nine which is exceeding the age-based standard value. Therefore, age was not taken into account for analysis.

Alternatively, anti-AQP4 Ab was detected only in 18.2% of the patients in the NMOSD group. This result was consistent with previous reports, the low prevalence of anti-AQP4 Ab positivity in SLE patients with brainstem spinal cord lesions.[11] From this result, autoantibodies other than anti-AQP4 Ab can cause NMOSD-like demyelination in patients with SLE. In the NMOSD-like demyelination group, QAlb was elevated, which was almost similar to that in the atypical NMOSD group. However, no significant difference in CSF IL-6 levels was observed between the atypical NMOSD and NMOSD-like demyelination groups. This observation may suggest that in both conditions a final common pathway involving IL-6 production exists. The only difference between those patients may depend on the timing of diagnosis when detected as neurological manifestations since the onset of the disease.

Though we suggest the feasibility of CSF IL-6 as a surrogate marker in this study, the therapeutic possibility of targeting IL-6 has been reported in patients with NMOSD. This study also showed a possible correlation between serum IL-6 and QAlb, although the difference was not statistically significant. Serum IL-6 was significantly correlated with QAlb in patients with diffuse NPSLE.[35] We speculate that in patients with SLE with NMOSD-like CNS lesions, serum IL-6 may also be involved in the pathogenesis. In a phase 3, randomized, double-blind, placebo-controlled trial, satralizumab, a monoclonal Ab against IL-6 receptors,

could reduce the flare-up risk of NMOSD compared with the placebo group.[36,37] Our results suggest CSF IL-6 as a possible surrogate marker for SLE complicated with NMOSD which may also suggest the utility of IL-6 inhibition in these patients.

This study has some limitations. First, the number of patients we recruited was small to conduct an accurate analysis. Secondly, retrospective data collection and lack of clinical, serological, and imaging follow-up made it difficult to evaluate pre- and post-treatment trends and the correlation between autoantibodies and CSF findings. Further studies should be conducted to reveal the association between NMOSD and NPSLE.

In conclusion, CSF IL-6 could be a useful surrogating biomarker for the disease activity of brainstem and/or spinal cord lesions in patients with SLE. Autoantibodies may contribute to the development of brainstem and/or spinal cord lesions in SLE from the viewpoint that autoantibodies mediate BBB rupture and demyelination, which are common in NMOSD.

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Declaration of conflicting interests

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors would like to thank you to Enago (www.enago.jp) for the English language review.

Author Contributions

YH wrote the manuscript. All authors contributed to the study design, to the collection of data, and to the interpretation of the results. YA mainly contributed to the conception and design of the study and the writing of the manuscript. KY critically revised the article for important intellectual content. All authors read, commented upon, edited various drafts, and approved the final version of the manuscript.

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Data availability

The data that support the findings of this study are displayed in the article and in the supplementary material. Others are available from the corresponding author upon reasonable request.

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Table 1. Baseline Patient Characteristics

	NMOSD-like CNS lesions (n = 12)	Diffuse NPSLE (n = 12)	*P* value
Patients Characteristics			
Age [years, median (range)]	40.0 (18–57)	39.0 (17–51)	0.400
Gender (male: female)	0:12	1:11	>0.999
Disease duration [month, median (range)]	57.0 (0–327)	2.0 (0–231)	0.032
Follow-up duration [month, median (range)]	32.5 (0–193)	111.0 (40–161)	0.015
Clinical Characteristics			
Flare of NPSLE	3 (25.0%)	0 (0.0%)	0.217
Fatal prognosis	3 (25.0%)	1 (8.3%)	0.590
SLEDAI [median (range)]	13 (0–24)	19.5 (8–30)	0.037
Symptoms			
Fever	2 (16.7%)	6 (50.0%)	0.193
Skin rash	0 (0.0%)	5 (41.7%)	0.037
Arthritis	4 (33.3%)	3 (25.0%)	>0.999
Pancytopenia	2 (16.7%)	5 (41.7%)	0.371
Pleuritis	1 (8.3%)	2 (16.7%)	>0.999
Pericarditis	0 (0.0%)	1 (8.3%)	>0.999
Lupus nephritis	4 (33.3%)	5 (41.7%)	>0.999
Sensory disturbance	9 (75.0%)	0 (0.0%)	<0.001

Muscle weakness	7 (58.3%)	0 (0.0%)	0.005
Bladder and rectal disturbance	4 (33.3%)	0 (0.0%)	0.093
Hiccup	2 (16.7%)	0 (0.0%)	0.478
Optic neuritis	0 (0.0%)	0 (0.0%)	>0.999
Delirium	1 (8.3%)	1 (12.5%)	0.155
Psychosis	0 (0.0%)	5 (41.7%)	0.037
Seizure	0 (0.0%)	3 (25.0%)	0.217
Mood disorder	0 (0.0%)	2 (16.7%)	0.478
Cognitive dysfunction	0 (0.0%)	2 (16.7%)	0.478

SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; NMOSD, neuromyelitis optica spectrum disorder

Table 2. Laboratories in patients.

Patients with SLE with brainstem and/or spinal cord lesions	NMOSD-like CNS lesions (n = 12)	Diffuse NPSLE (n = 12)	*P* value
Laboratories			
Serum			
Hypocomplementemia	8 (66.7%)	10 (83.3%)	0.640
Anti-dsDNA	8 (66.7%)	9 (75.0%)	>0.999
Anti-Sm	6 (50.0%)	10 (83.3%)	0.193
Antiphospholipid	7 (58.3%)	5 (41.7%)	0.684
Anti-ribosomal P	3 (33.3%)	6 (50.0%)	0.400
Anti-GluN2	3 (27.3%) (n = 11)	8 (72.7%) (n = 11)	0.086
Anti-AQP4	2 (18.2%) (n = 11)	-[†]	-
Anti-MOG	0 (0.0%) (n=8)	-[†]	>0.999
Pretreatment serum IL-6 [pg/mL, median (range)]	4.6 (0-19.0) (n=9)	3.9 (0.4-37.2) (n=7)	>0.999
Cerebrospinal fluid	(n=11)		
Cell number (0-5) [cells/μL, median (range)]	3.0 (0–154)	0.0 (0–45)	0.023
TP (10-40) [mg/mL, median (range)]	77.0 (24–980)	41.5 (26–152)	0.164

MBP (<313) [pg/mL, median (range)]	135.1 (0–3230) (n = 9)	0.0 (0–114) (n = 6)	0.120
IgG index (<0.73) [median (range)]	0.77 (0.41–1.00) (n = 8)	0.60 (0.25–1.01) (n = 8)	0.982
Oligoclonal band positive	3 (33.3%) (n = 9)	1 (25.0%) (n = 4)	>0.999
QAlb	11.5 (4.1–116.4) (n = 9)	30.6(5.6–32.2) (n = 3)	0.464
Pretreatment CSF IL-6 [pg/mL, median (range)]	29.1 (1.9–15800)	10.0 (3.6–611)	>0.999
Posttreatment CSF IL-6 [pg/mL, median (range)]	3.8 (1.9–65) (n = 8)	3.2 (1.2–5.9)	0.254

SLE, systemic lupus erythematosus; NMOSD, neuromyelitis optica spectrum disorder; anti-dsDNA, anti-double strand deoxyribonucleic acid antibody; anti-Sm, anti-Smith antibody; antiphospholipid, antiphospholipid antibody; anti-ribosomal P, anti-ribosomal P antibody; anti-GluN2, anti-glutamate receptor subunit 2 antibody; anti-AQP4, anti-Aquaporin4 antibody; anti-MOG, Anti-myelin-oligodendrocyte glycoprotein antibody; CSF, cerebrospinal fluid; TP, total protein; MPB, myelin basic protein; QAlb, CSF/serum albumin quotient × 1,000

†: No measurement because these were not case of NMOSD

Table 3. Baseline Patient Characteristics in patients with NMOSD-like CNS lesions

	Atypical NMOSD (n = 8)	NMOSD-like demyelination (n = 4)	*P* value
Patients Characteristics			
Age [years, median (range)]	35.0 (18–57)	47.0 (40–52)	0.097
Gender (male: female)	0:8	0:4	>0.999
Disease duration [month, median (range)]	59.0 (0–280)	54.0 (115–327)	0.109
Follow-up duration [month, median (range)]	85.0 (0–160)	13.0 (10–56)	0.539
Clinical Characteristics			
Flare of NPSLE	3 (37.5%)	0 (0.0%)	0.491
Fatal prognosis	3 (37.5%)	0 (0.0%)	0.491
SLEDAI [median (range)]	14 (0–24)	8 (4–13)	0.301
Symptoms			
Fever	2 (25.0%)	0 (0.0%)	0.515
Skin rash	0 (0.0%)	0 (0.0%)	>0.999
Arthritis	4 (50.0%)	0 (0.0%)	0.208
Pancytopenia	1 (12.5%)	1 (25.0%)	>0.999
Pleuritis	1 (12.5%)	0 (0.0%)	>0.999
Lupus nephritis	3 (37.5%)	1 (25.0%)	>0.999
Sensory disturbance	7 (87.5%)	2 (50.0%)	0.236
Muscle weakness	5 (62.5%)	2 (50.0%)	>0.999

Bladder and rectal disturbance	4 (50.0%)	0 (0.0%)	0.208
Hiccup	2 (25.0%)	0 (0.0%)	0.515
Optic neuritis	0 (0.0%)	0 (0.0%)	>0.999
Delirium	1 (12.5%)	0 (0.0%)	>0.999

SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; NMOSD, neuromyelitis optica spectrum disorder

Table 4. Laboratories in patients with brainstem and/or spinal lesions in patients with NMOSD-like CNS lesions

Patients with SLE with brainstem and/or spinal cord lesions	Atypical NMOSD (n = 8)	NMOSD-like demyelination (n = 4)	*P* value
Laboratories			
Serum			
Hypocomplementemia	6 (75.0%)	2 (50.0%)	>0.999
Anti-dsDNA	5 (62.5%)	3 (75.0%)	0.548
Anti-Sm	5 (62.5%)	1 (25.0%)	0.546
Antiphospholipid	4 (50.0%)	3 (75.0%)	0.576
Anti-ribosomal P	2 (25.0%)	1 (25.0%)	>0.999
Anti-GluN2 (n = 10)	1 (16.7%) (n = 6)	1 (25.0%)	>0.999
Anti-AQP4	2 (28.6%) (n = 7)	0 (0.0%)	0.515
Anti-MOG	0 (0.0%) (n=6)	0 (0.0%) (n=2)	>0.999
Pretreatment serum IL-6 [pg/mL, median (range)]	2.5 (0-19) (n=5)	5.3 (2.9-6.1) (n=4)	0.730
Cerebrospinal fluid (n=11)	(n = 7)	(n = 4)	
Cell number (0-5) [cells/μL, median (range)]	4.0 (0–154)	2.5 (0–6)	0.242
TP (10-40) [mg/mL, median (range)]	104.0 (30–980)	61.0 (24–77)	0.164

MBP (<313) [pg/mL, median (range)] (n = 9)	135.1 (0–3230) (n = 6)	62.2 (0–573) (n = 3)	0.929
IgG index (<0.73) [median (range)] (n = 8)	0.84 (0.41–1.00) (n = 4)	0.64 (0.55–0.94)	0.686
Oligoclonal band positive (n = 9)	2 (40.0%) (n = 5)	1 (25.0%)	>0.999
Q-albumin (n = 9)	16.7 (4.9–116.4) (n = 5)	7.65 (4.1–11.5)	0.127
Pretreatment CSF IL-6 [pg/mL, median (range)]	8.3 (1.9–15800)	34.1 (3–377)	0.412
Posttreatment CSF IL-6 [pg/mL, median (range)] (n = 8)	5.9 (2–65) (n = 5)	2 (1.9–5) (n = 3)	0.161

SLE, systemic lupus erythematosus; NMOSD, neuromyelitis optica spectrum disorder; anti-dsDNA, anti-double strand deoxyribonucleic acid antibody; anti-Sm, anti-Smith antibody; antiphospholipid, antiphospholipid antibody; anti-ribosomal P, anti-ribosomal P antibody; anti-GluN2, anti-glutamate receptor subunit 2 antibody; anti-AQP4, anti-Aquaporin4 antibody; anti-MOG, Anti-myelin-oligodendrocyte glycoprotein antibody; CSF, cerebrospinal fluid; TP, total protein; MPB, myelin basic protein; Q-albumin, CSF/serum albumin quotient × 1,000

Table 5. MRI findings, symptom, treatment and prognosis in patients with NMOSD-like CNS lesions

Case	MRI findings	Neurological findings			Treatment				
			Distribution						
		Manifestations	Sensory	Motor	Steroid pulse therapy	Immunosuppressants	Prognosis	Neurological improvement	Recurrence
	Atypical NMOSD group								
1	TH11 to medullary cone	Sensory disturbance Paraplegia Bladder and rectal disturbance	Both lower limbs	Both lower limbs	+	MMF	Surviving	Remaining bladder disturbance	-
2	C7 and TH3 to TH8	Sensory disturbance Paraplegia Bladder and rectal disturbance	TH5–TH6 and below	Both lower limbs	+	IVCY	Demise	NA	NA
3	Medulla oblongata,	Sensory disturbance Vertigo	Right lower limbs	None	+	None	Surving	Improvement	+

	C2 to TH1	Double vision, hiccup					
4	TH4 to TH12	Paraplegia Sensory disturbance Bladder and rectal disturbance	Abdomen and both lower limbs	Both lower limbs +	IVCY、IVIg	Surviving	Remaining paraplegia +
5	TH4 to TH 7	Muscle weakness Bladder and rectal disturbance	None	Both lower limbs +	IVCY、IVIg	Demise	NA +
6	TH2 to TH4	Sensory disturbance	TH3 and below, upper limb	None +	None	Surviving	Improvement -
7	Medulla to C6	Nausea Hiccup Sensory disturbance	Face, both upper limbs, nipple	None -	None	Demise	NA NA
8	Pons and cerebral cortex	Dysarthria Sensory disturbance Muscle weakness	Chest and below, both lower	Upper left limb, both lower +	IVCY, CyA	Surviving	Remaining consciousness due to cerebral -

			limbs	limbs				hemorrhage
	NMOSD-like demyelination							
9	Midbrain and pons	Double vision	None	None	+	None	Surviving	Improvement -
10	C5 to C6	Sensory disturbance	Back and both upper and lower limbs	None	-	HCQ	Surviving	Improvement -
11	Pons	Vertigo, paralysis	None	Left side of the body	+	None	Surviving	Remaining paralysis -
12	C6	Muscle weakness	None	Right upper and lower limbs	+	None	Surviving	Improvement -

Figure 1. Changes of MRI findings in patients with NMOSD-like CNS lesions (T2-weighted imaging).

The images are representative and each number refers to Supplementary table 3. (a) High-intensity area observed in the medullary cone on TH11 level. (b) Case 3, Multiple high-intensity area observed in brain stem and cervical spinal cord. Both cases showed that high-intensity area was reduced and paler with treatment.

NMOSD, neuromyelitis optica spectrum disorder.

Figure 2. Change in CSF IL-6 levels in patients with NPSLE.

In twelve patients with NMOSD-like CNS lesions where we could perform follow-up tests, the CSF IL-6 levels before treatment significantly decreased (P=0.008) after treatment (a). In twelve patients with diffuse NPSLE also showed a significant decrease in CSF IL-6 before and after treatment (P=0.001) (b). These data are analyzed using Wilcoxon's signed-rank test.

CSF, cerebrospinal fluid; IL-6, interleukin-6; NMOSD, neuromyelitis optica spectrum disorder. CNS, central nervous system; NMOSD-like CNS lesions, patients with SLE who had optic neuritis, brainstem or spinal cord lesions regardless of NMOSD classification criteria; the diffuse NPSLE without remarkable MRI abnormality.

Figure 3. Changes in CSF IL-6 levels in patients with NMOSD-like CNS lesions.

Follow-up study was performed in 8 patients, including 5 patients with atypical NMOSD and 3 patients with NMOSD-like demyelination. Comparison of the CSF IL-6 levels before and after treatment in patients with atypical NMOSD (a) and in patients with NMOSD-like demyelination (b) by Wilcoxon's signed-rank test.

CSF, cerebrospinal fluid; IL-6, interleukin-6; NMOSD, neuromyelitis optica spectrum disorder. Atypical NMOSD: patients with SLE who had LETMs and/or brainstem lesions, which fulfilled the international criteria for definite NMOSD. NMOSD-like demyelination: patients who had brainstem and/or spinal lesions without NMOSD.

Figure 4. Correlations between serum IL-6 and serum anti-dsDNA, anti-Sm, QAlb, and pretreatment CSF IL-6 in patients with NMOSD-like CNS lesions.

These data were calculated using Spearman's rank correlation coefficient. There was no correlation between serum IL-6 and autoantibodies or CSF findings.

Anti-dsDNA, anti-double strand deoxyribonucleic acid antibody; anti-Sm, anti-Smith antibody; QAlb, CSF/serum albumin ratio; CSF, cerebrospinal fluid; IL-6, interleukin-6; NMOSD, neuromyelitis optica spectrum disorder; CNS, central nervous system.

Figure 5. Correlations between pretreatment CSF IL-6 and serum anti-dsDNA, anti-Sm, and QAlb in patients with NMOSD-like CNS lesions.

These data were calculated using Spearman's rank correlation coefficient. Serum anti-dsDNA correlated with pretreatment CSF IL-6 (a). No correlation was found between anti-Sm and pretreatment CSF IL-6 (b). Anti-dsDNA did not correlate with QAlb (c). A correlation between serum anti-Sm and QAlb was observed (d).

Ab, antibody; CSF, cerebrospinal fluid; IL-6, interleukin-6; NMOSD, neuromyelitis optica spectrum disorder; CNS, central nervous system; anti-dsDNA, anti-double strand deoxyribonucleic acid antibody; anti-Sm, anti-Smith antibody; QAlb, CSF/serum albumin ratio.

Figure 6. Correlations between pretreatment CSF IL-6 and serum anti-dsDNA, anti-Sm, and QAlb in patients with atypical NMOSD.

These data were calculated using Spearman's rank correlation coefficient. Anti-dsDNA seemed to be correlated with CSF IL-6 and QAlb (a, c); however, this correlation was not statistically significant. A correlation between serum anti-Sm and pretreatment CSF IL-6 and QAlb was observed (b, d). For CSF IL-6 and QAlb, only pretreatment CSF IL-6 was correlated (e).

Ab, antibody; CSF, cerebrospinal fluid; IL-6, interleukin-6; NMOSD, neuromyelitis optica spectrum disorder; anti-dsDNA, anti-double strand deoxyribonucleic acid antibody; anti-Sm, anti-Smith antibody; QAlb, CSF/serum albumin ratio; Atypical NMOSD: patients with SLE who had LETMs and/or brainstem lesions, which fulfilled the international criteria for definite NMOSD.

Figure 7. Correlations between serum IL-6 and other markers. Correration of serum IL-6 and serum anti-dsDNA

(a), anti-Sm (b), Q-albumin (c), and pretreatment CSF IL-6 (d) in patients with atypical NMOSD by Spearman's rank correlation coefficient. Anti-dsDNA, anti-double strand deoxyribonucleic acid antibody; anti-Sm, anti-Smith antibody; Q-albumin, CSF/serum albumin quotient x 1,000.

Figure 1.

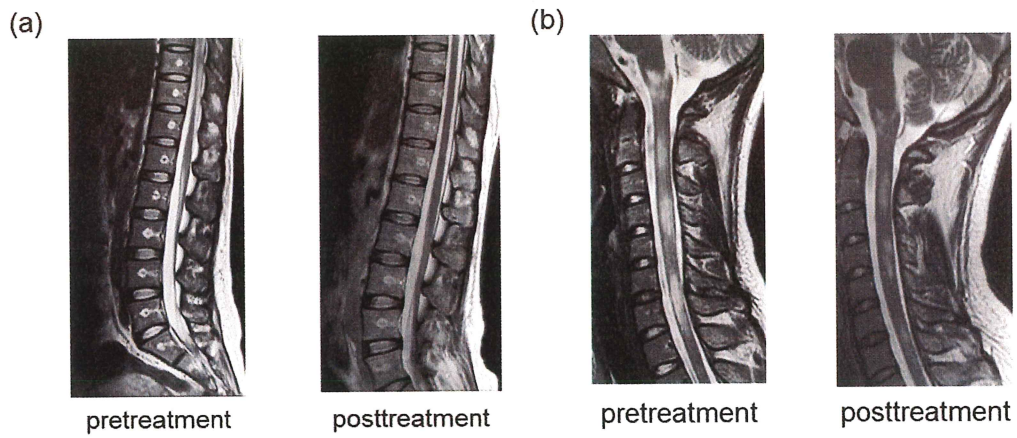


Figure 2.

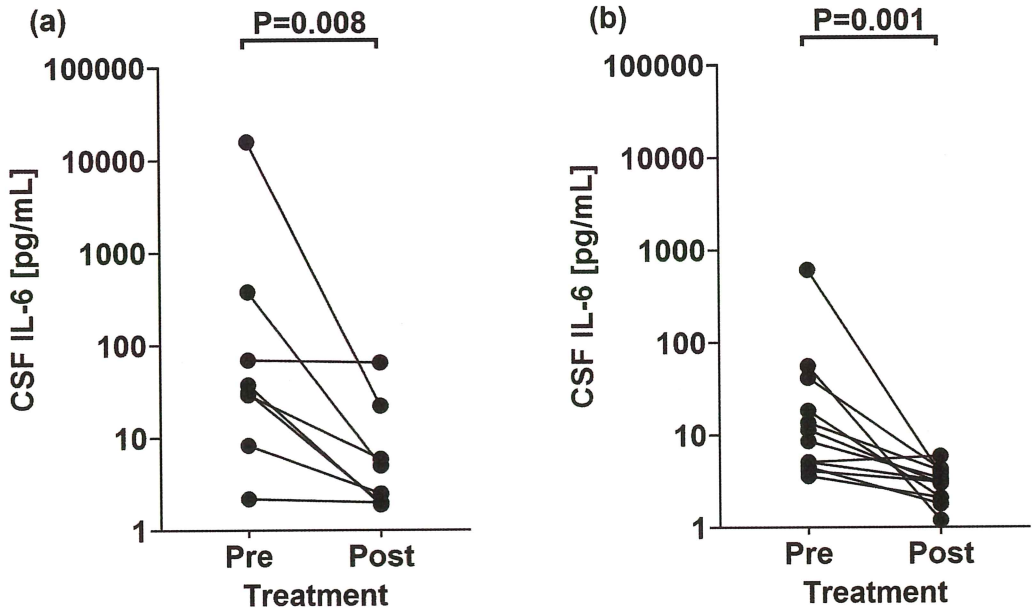


Figure 3.

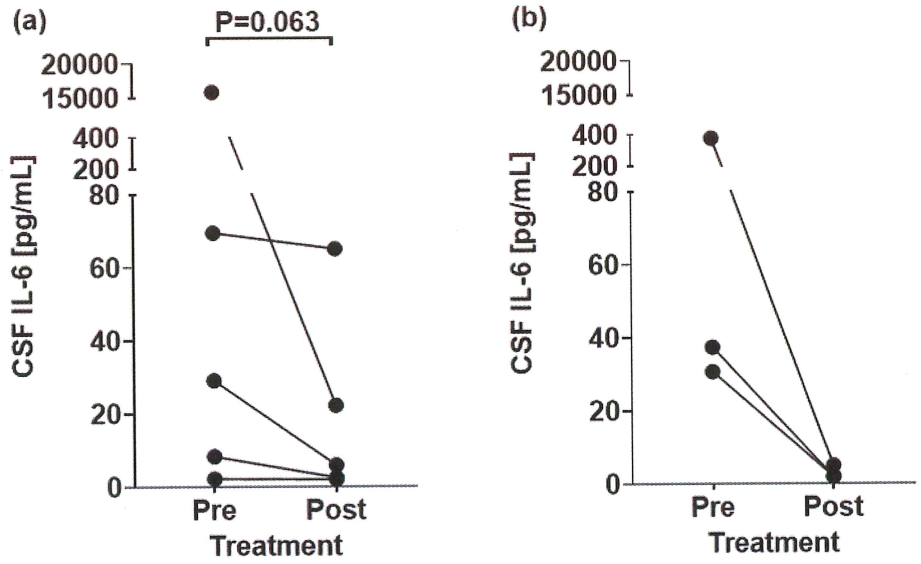


Figure 4.

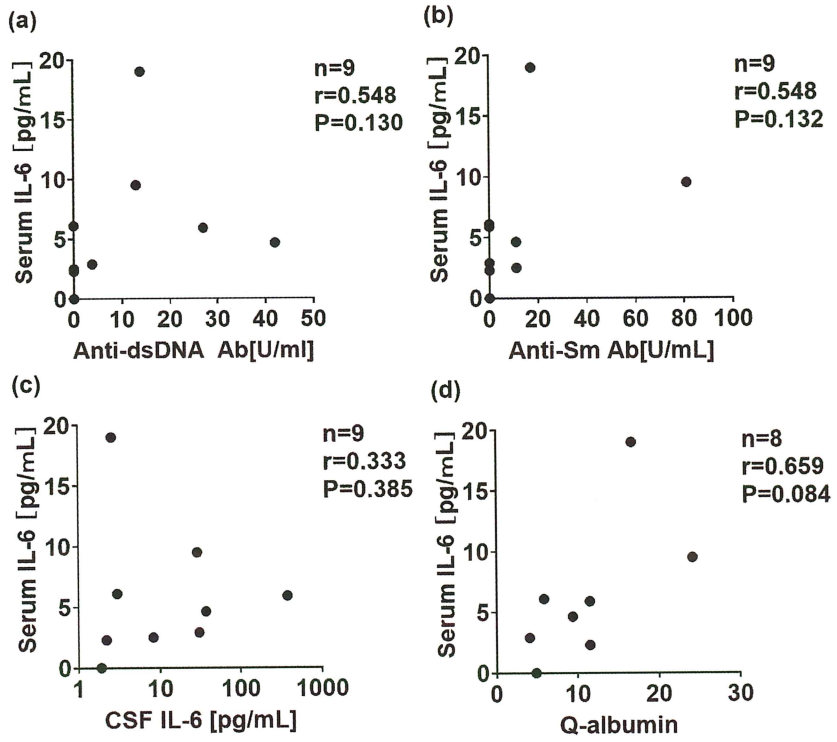


Figure 5.

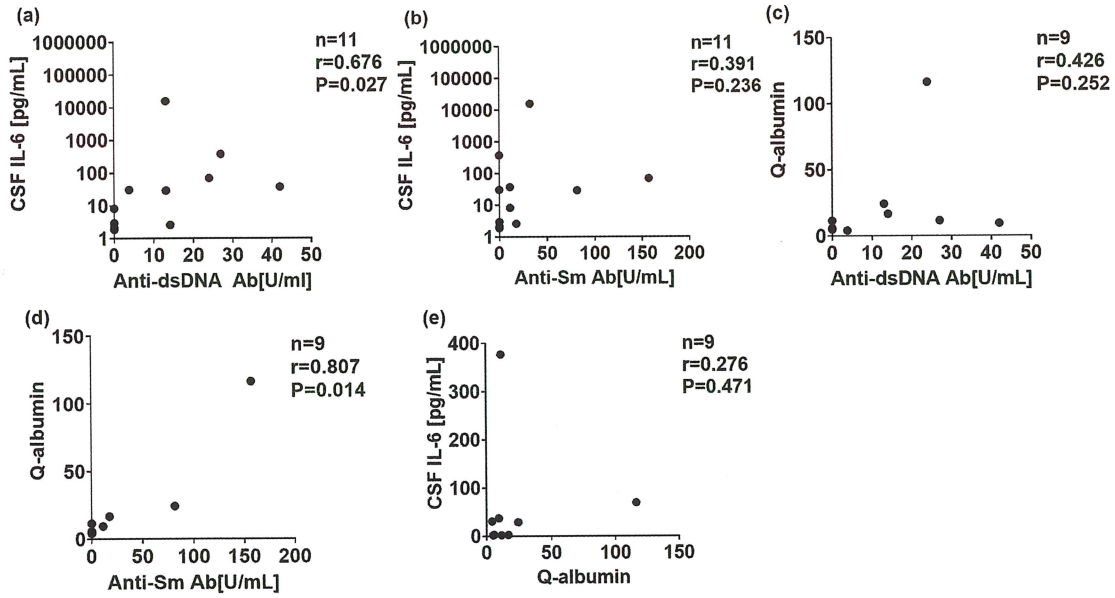


Figure 6.

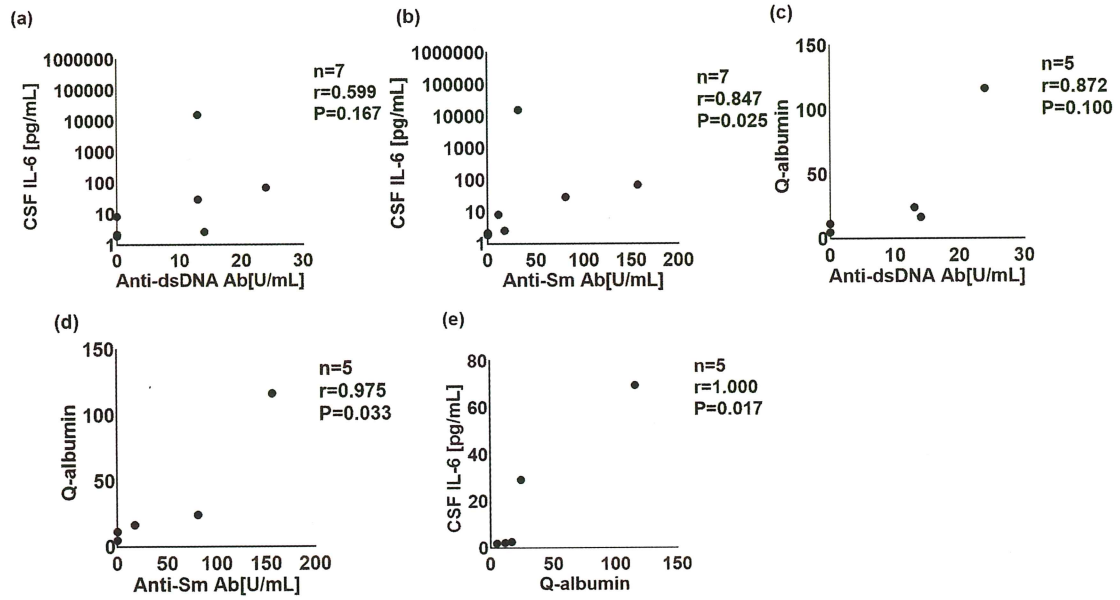
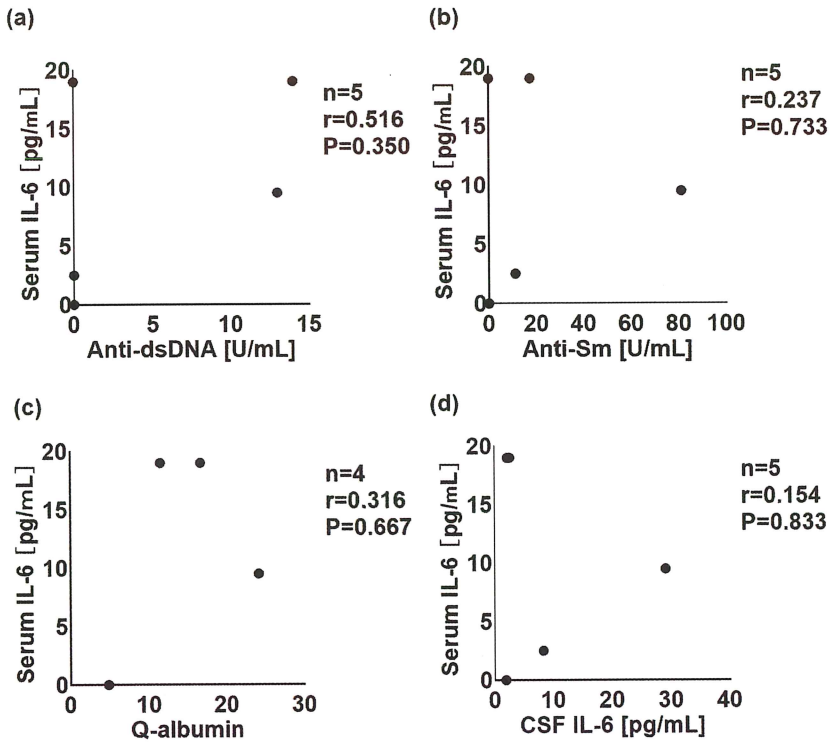


Figure 7.


(a)
Serum IL-6 [pg/mL]
Anti-dsDNA [U/mL]
n=5
r=0.516
P=0.350
(b)
Serum IL-6 [pg/mL]
Anti-Sm [U/mL]
n=5
r=0.237
P=0.733
(c)
Serum IL-6 [pg/mL]
Q-albumin
n=4
r=0.316
P=0.667
(d)
Serum IL-6 [pg/mL]
CSF IL-6 [pg/mL]
n=5
r=0.154
P=0.833