Brief communication

Distinguishing the cerebrospinal fluid cytokine profile in neuropsychiatric systemic lupus erythematosus from other autoimmune neurological diseases

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ABSTRACT

Neuropsychiatric systemic lupus erythematosus (NPSLE) is a serious complication in SLE. Although the mechanism of NPSLE remains unclear, cytokine and chemokines are considered to be involved in their pathogenesis. Here we used Bio-Plex Pro assays to examine 27 types of cytokines and chemokines in the cerebrospinal fluid (CSF) of 32 NPSLE patients. We used the CSF of 20 patients with multiple sclerosis (MS) and 22 patients with neuromyelitis optica (NMO) as a disease control group. Fourteen of 27 cytokines/chemokines were significantly higher in the NPSLE patients compared to the MS/NMO patients. We could identify six “minimum predictive markers” by using a weighted-voting algorithm that could distinguish NPSLE from MS and NMO: interleukin (IL)-17, IL-2, interferon (IFN)-γ, IL-5, basic fibroblast growth factor (FGF)-basic and IL-15. The determination of various types of CSF cytokine profiles may contribute to the diagnosis of NPSLE and may help elucidate the mechanisms underlying this disease.

1. Introduction

Neuropsychiatric systemic lupus erythematosus (NPSLE) syndromes involve both the central and peripheral nervous systems. Despite advances in the understanding of the immunopathogenic and clinical aspects of SLE, NPSLE remains a diagnostic and therapeutic challenge [1]. Cytokines and chemokines are considered biomarkers and therapeutic targets in NPSLE. Of note, abnormalities in cerebrospinal fluid (CSF) have been reported in patients with NPSLE. Increased levels of proinflammatory cytokines and chemokines have been reported in the CSF of patients with NPSLE, including cytokines such as interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), C-C motif ligand (CCL)2/monocyte chemoattractant protein-1 (MCP-1) and C-X-C motif ligand (CXCL) 10/inducible protein-10 (IP-10) [2, 3]. Among these, CSF IL-6 is major cytokine for the diagnosis of NPSLE. IL-6 is a proinflammatory cytokine secreted by immune cells and activated astrocytes, with a wide variety of functions. The sensitivity and specificity of the diagnosis of lupus psychosis were 87.5% and 92.3%, respectively, at the cut-off value of 4.3 pg/mL [4].

Multiple sclerosis (MS) and neuromyelitis optica (NMO) are chronic autoimmune inflammatory diseases affecting the central nervous system (CNS). Disruption of the blood-brain barrier (BBB) is a known mechanism of the disease

process in these two CNS diseases. MS and NMO are also considered T cell-mediated autoimmune diseases, and both the Th1/Th2 balance and Th17 cells play an important role in the pathogenesis [5]. Elevated CSF IL-6 and IL-8 levels in NMO patients have also been reported [6]. CSF IL-6 and IL-8 levels are significantly higher in patients with NMO than in patients with MS [6]. Similar cytokines/chemokines have been evaluated in NPSLE, MS and NMO but the therapeutic strategy and management are quite different among these three diseases.

In this study we evaluated multiple cytokines, chemokines and growth factors in NPSLE compared to MS and NMO as disease controls. We found a specific combination of cytokines, chemokines and growth factors in NPSLE that can be distinguished from the profiles of the other two diseases. This analysis might help clarify the mechanism of NPSLE caused by inflammation and may provide an important resource for pharmaceutical developments.

2. Methods

2.1. Study design and patients

We studied 32 patients who were admitted to Nagasaki University Hospital in a 7-year period from 2006 through 2013 and fulfilled at least four of the 11 revised criteria of the

American College of Rheumatology (ACR) for the classification of SLE [7]; they were all diagnosed with NPSLE by rheumatologists and psychiatrists. Neuropsychiatric manifestations showing psychiatric symptoms such as mood disorder, anxiety disorder, psychosis, acute confusional state, or cognitive dysfunction were evaluated by a psychiatrist and classified according to the ACR nomenclature and case definitions for NPSLE [8].

All information about clinical symptoms and laboratory data were reviewed retrospectively using the patients' medical records. The patients' age, gender, clinical events, results of serum laboratory tests, the CSF analysis, brain magnetic resonance imaging (MRI), and single photon emission computed tomography (SPECT) and their diagnoses and treatment were all analyzed.

As disease controls, we used samples from 20 relapsing remitting MS (RRMS) patients, samples from 22 NMO patients, 11 normal pressure hydrocephalus (NPH) patients, and 16 viral meningitis (VM) patients from the Department of Clinical Neuroscience and Neurology, Nagasaki University Hospital. For the diagnosis of NMO, we defined NMO spectrum disorder (NMOSD) based on the revised NMO criteria [9]. All of the NMO patients were positive for anti-AQP4 antibodies in sera. CSF of NPH patients were used for non-autoimmune, non-inflammatory neurological controls and

VM patients were used for positive controls. The protocol was approved by the Institutional Review Board of the Nagasaki University Hospital.

2.2. Multiplex cytokine bead assay

We performed a multiplex cytokine bead assay using undiluted CSF supernatants and the Bio-Plex Pro Human Cytokine Group I 27-Plex Panel analyzed with a Bio-Plex® MAGPIX™ Multiplex Reader (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. CSF samples were centrifuged within 30 min at 1500 rpm at 4°C for 5 min, and the liquid phase of the CSF was stored at −80°C until use. The levels of 27 cytokines/chemokines and growth factors in the liquid phase of the CSF, namely, IL-1β, IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-5, IL-6, IL-7, CXCL8/IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, basic fibroblast growth factor (FGF)-basic, CCL11/eotaxin, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), IFN-γ, IP-10, CCL2/MCP-1, CCL3/macrophage inflammatory protein (MIP)-1α, CCL4/MIP-1β, platelet-derived growth factor (PDGF)-BB, CCL5/regulated on activation, normal T cell expressed and secreted (RANTES), TNF-α, and vascular endothelial growth factor (VEGF) were measured as described [10, 11].

The cytokines/chemokines/growth factors concentrations were calculated based on the respective standard curve for each cytokine/chemokine/growth factor concentration of the standards assayed in the same manner as the CSF samples. The detection limit for each molecule was determined by the recovery of the corresponding standard, and the lowest values with more than 70% recovery were set as the lower detection limits. All samples were analyzed in duplicate.

2.3. Construction of diagnostic systems

Next, we selected which of the 27 cytokines/chemokines/growth factors were useful markers for distinguishing NPSLE from MS and NMO. The weighted-voting (WV) algorithm was used as described [12, 13]. The ranking of the 27 cytokines/chemokines for this algorithm was based on the signal-to-noise ratio (SNR). For each marker set of 27 cytokines/chemokines/growth factors based on the absolute value of the SNR, we calculated the sensitivity, specificity, accuracy, positive predictive value, negative predictive value, and Matthews correlation coefficient (MCC). The MCC is used as a measure of the quality of binary (two-class) classifications. We defined the “distinction set” as a set that had a minimum number of markers with higher MCCs.

In the WV algorithm, each marker belonging to a predictor set is assigned a vote value, and prediction is based on the relative vote value in NPSLE and in MS and NMO. The vote value of marker 'a,' V_a, and the weight of marker 'a,' W_a, are calculated by the following two formulas:

$$v_a = w_a \times \left| x_a - \frac{\overline{X}_{na} + \overline{X}_{oa}}{2} \right|$$

$$w_a = \frac{\overline{X}_{na} - \overline{X}_{oa}}{S_{na} + S_{oa}}$$

Here, X_a is the expression level of each marker a in a CSF sample. $\bar{X}_{na}$ is the mean expression level of each marker 'a' in NPSLE in the learning set. $\bar{X}_{oa}$ represents the mean expression level in the other diseases (i.e., MS and NMO). S_{na} is the standard deviation in NPSLE, and S_{oa} represents the standard deviation in the others. The SNR used for ranking the markers is the absolute value of W_a; the diagnostic markers were selected in descending order of SNR. We selected 'i' pieces of markers, in the order of the absolute number of SNR, and we defined the prediction strength (PS) value of the i pieces of markers using the following formula:

$$\text{Prediction Strength Value} = \frac{\sum_{n=1}^{i} v_a}{\sum_{n=1}^{i} \left| v_a \right|}$$

In the case of missing values, the vote of the gene is assigned as 0. PS is defined in which positive and negative PS indicates the prediction of others and NPSLE, respectively.

2.4. Statistical analysis

We used a t-test and the nonparametric Wilcoxon rank sum test for the inter-group comparisons of multiple variables. The Spearman rank correlation coefficient was used for the analyses of correlations between cytokines, chemokines, and growth factors in the patients with NPSLE, MS and NMO. All of the statistical analyses were performed using JMP® Pro10 (SAS Institute, Cary, NC, USA) and R Statistical Software (Foundation for Statistical Computing, Vienna, Austria). The significance level was set at $p<0.05$.

3. Results

3.1. Demographic and disease-related variables

Supplementary Table 1 shows the demographic and disease-related characteristics of the 32 NPSLE patients at examination. Thirty-one of the 32 patients (96.9%) were females. The median age at the onset of NPSLE was 34.9 yrs, ranging from 15 to 50 yrs. The

median duration from SLE onset to first neuropsychiatric event was 8.7 yrs. The median SELENA-SLEDAI score at the disease onset of NPSLE was 13.3. The median levels of anti-ds-DNA antibodies (U/mL), C3 (mg/dL) and C4 (mg/dL) were 20.2, 86.3 and 19.4, respectively. The median level of CSF IgG index was 0.61. Seven (21.9%) of the NPSLE patients also fulfilled the criteria for the antiphospholipid syndrome. Eight (25.0%) of the patients had anti-ribosomal P antibodies, and 17 (53.1%) patients had abnormal MRI findings.

A total of 39 neuropsychiatric manifestations were observed in the 32 NPSLE patients. Nine of the 19 ACR Ad Hoc Committee classifications of NPSLE manifestations [2] were identified in this study (Suppl. Table 2). Nine of the 32 (28.1%) patients presented more than one NPSLE manifestation. Most of the NPSLE manifestations (97.4%) were in the CNS. The most frequent manifestation was headache (35.9%), followed by psychosis (25.6%) and mood disorder (12.8%).

3.2. Comparison of cytokine/chemokine/growth factor levels

We analyzed 32 NPSLE patients, 20 RRMS patients, samples from 22 patients with NMO, 11 NPH patients and 16 VM patients. The representative results are shown in Supplementary Figure 1. Although some of the cytokine and chemokine levels of the

VM patients were extremely high compared to the other groups, we did not observe any significant differences among the NPSLE, MS and NMO groups. We therefore referred to a previous report [14] and compared the results among only the present NPSLE, MS and NMO groups. Since there was no significant difference between the MS and NMO groups (Fig. 1), we speculated that it is problematic to distinguish MS and NMO, and we thus compared NPSLE and the “others” (i.e., the combined group of MS and NMO patients).

Fourteen of the 27 cytokines/chemokines/growth factors were significantly higher in the NPSLE group compared to the others (Table 1). CSF IL-6 and IL-8 levels, which had been reported to be increased in NPSLE and NMO, were not significantly different between the present NPSLE group and the others. Only the CSF level of IL-1β was lower in the NPSLE group compared to the others.

3.3. Ranking of the cytokines/chemokines/growth factors for diagnosing NPSLE

Our ranking of the cytokines/chemokines/growth factors for diagnosing NPSLE determined by the weighted-voting algorithm showed that the combination of IL-17, IL-2, IFN-γ, IL-5, FGF-basic and IL-15 had the highest MCC (88.99%). According to this method, the accuracy of discriminating a responder from a non-responder was

94.59% (Table 2). The WV values (v_a) of each of these six markers and the PS value were obtained by the formulas shown in Supplementary Table 3; X_a is the expression level of each marker ‘a’ in a CSF sample.

4. Discussion

NPSLE is still diagnosed based on a combination of clinical observations, laboratory tests and imaging techniques, because there are no specific markers for NPSLE. Multiple cytokines and chemokines have been implicated in the pathophysiology of NPSLE. Previous studies obtained evidence of the intrathecal production of IL-6 [3, 4] and other cytokines including IL-8 [3, 15], IL-10 [16], TNF-α [16] and IFN-γ [17] in patients with NPSLE. Cytokines associated with NPSLE are produced by neuronal and glial cells, probably in response to autoantibodies within the intrathecal space.

MS and NMO, chronic autoimmune inflammatory diseases affecting the CNS, are sometimes difficult to distinguish from NPSLE. An overlap diagnosis often referred to as lupoid sclerosis has been described [18]. In SLE, MS and NMO, the BBB is pivotal. There is evidence implicating BBB damage as an important component in the development of NPSLE, MS and NMO, occurring through damage to the barrier’s integrity by environmental triggers such as cytokines, chemokines and growth factors.

For example, the stimulation of human brain microvessel endothelial cells (HBMECs) with cytokines such as IL-1β, IL-8, TNF- α, and IFN-γ is known to induce increased permeability of the monolayers [19, 20]. Additionally, CSF from NPSLE patients with IL-6 and IL-8 was correlated with MMP-9 levels, and the latter is associated with degradation of the BBB extracellular matrix [21]. In both an *in vitro* investigation and in $CCR2^{-/-}$ mice, CCL2 signaling appeared to play a role in BBB disruption [22, 23].

We found that 14 of the 27 CSF cytokines/chemokines/growth factors used in the present study were significantly higher in the NPSLE group compared to the others (Table 1). In addition, we measured the serum markers of NPSLE, MS and NMO at nearly the same time that the lumbar puncture was performed. We found that serum IL-15 and IFN-γ were significantly increased in the non-NPSLE patients compared to the NPSLE patients (Suppl. Table 4). It is interesting that these results were the inverse of the CSF cytokines' results, and these results might indicate that BBB disruption is much stronger in NPSLE compared to non-NPSLE.

CSF IL-6 is a major cytokine for the diagnosis of NPSLE because of its sensitivity and specificity in certain conditions [4]. Elevated CSF IL-6 levels in NPSLE and NMO patients have been reported [3, 6]. However, our present findings showed that

there was no significant difference in the CSF IL-6 levels among the NPSLE, MS and NMO patients (data not shown). We speculate that CSF IL-6 is involved to some extent in the pathogenic mechanism of all of these autoimmune neurological diseases, and thus CSF IL-6 was not a marker that could be used to distinguish them.

Our study shows for the first time identified predictive markers in the CSF of NPSLE patients compared to MS and NMO patients. Among them, IL-17, IL-2, IL-5, FGF-basic and IL-15 have not been well documented in NPSLE. There has been an increasing focus on the role of Th17, which might be a promising therapeutic target for SLE. CD3+CD4(−)CD8(−) (double-negative) T cells are an important source of IL-17 in SLE. IL-17 produced by double-negative and CD4 T cells participates in the pathogenesis of SLE [24]. IL-17 promotes the production of IL-6 [25] and other inflammatory mediators by endothelial cells, fibroblasts, macrophages and astrocytes [26] .

IL-2, a cytokine with multifaceted effects, is important for immune cell activation and peripheral tolerance. The therapeutic efficacy of IL-2 in SLE has been suggested [27, 28]. In another study, no association with IL-2 was found in NPSLE patients who had neurological symptoms without evidence of a neurological disease [29]. However, our patients' backgrounds are different from those of the patients in that

study; we compared NPSLE patients with those with other autoimmune neurological disorders. The meaning of elevated IL-2 in NPSLE is requires further investigation.

As with IL-2, IL-15 can expand T-cell populations, and IL-15-deficient mice have greatly reduced numbers of lymphocytes [30]. Moreover, IL-15 can promote excessive antibody production in B cells [31]. Increased levels of serum IL-15 has also been observed in patients with SLE, and the IL-2R are correlated with markers that are potential targets of IL-15 [32].

The possible role of IL-5 in the pathogenesis of lupus has not been extensively evaluated. Several studies suggested that abnormally high levels of IL-5 may have a role in the abnormal expansion of auto-reactive B-1 cells and the subsequent suppression of autoimmune disease [33]. Additionally, elevated IL-5 mRNA has been reported in cutaneous lupus erythematosus [34]. The authors of those studies suggested that IL-5, produced by the Th2 subset of CD4+ T cells may contribute the development of autoimmunity in lupus. However, the potential relevance of these results to NPSLE is unclear.

FGF-basic is a potent angiogenic factor whose activity is involved in endothelial cell and fibroblast survival, proliferation, migration, and tube formation, together with

VEGF [35]. An increased FGF-basic level was reported in lupus and tended to be correlated with disease activity [36].

There has been no thorough comparison of the CSF of NPSLE, MS and NMO patients, to the best of our knowledge. Here, we found that NPSLE patients showed significantly higher levels of cytokines, chemokines and growth factors compared to MS and NMO patients. Among these factors, IL-17, IL-15, IL-9, IL-5, IL-12 p70, IL-7, FGF-basic, eotaxin, IL-13, GM-CSF and VEGF had not been observed in the CSF of NPSLE patients. In the present examination, we were able to detect cytokines, chemokines and growth factors with the Bio-plex assay, even if they were present in very small amounts. Since almost all of the above markers had already been shown elsewhere to be increased in MS or NMO, it would be difficult to distinguish MS or NMO from NPSLE using the above markers. We therefore devised a numerical prediction scoring system that clearly separated the NPSLE patients from the other patients. We identified six “minimum predictive markers” — IL-17, IL-2, IFN-γ, IL-5, FGF-basic and IL-15 — by a weighted-voting algorithm that showed the highest MCC and predicted the test set with 94.59% accuracy.

These predictive markers seem not to be correlated with each other; however they certainly involve Th1, Th2 and Th17 cells. This may indicate that these six markers

contribute more strongly to induce both pro-inflammatory and antibody-mediated neuronal and glial cells' destruction in NPSLE compared to MS and NMO, and we suspect that they are essential for the pathogenesis of NPSLE.

Focusing on other markers is also important. We found that the correlations of pairs of cytokines/chemokines/growth factors showed different patterns in NPSLE, MS and NMO. The correlation coefficients of the cytokines/chemokines were the most significant: PDGF-BB, MIP-1a, MIP-1b and RANTES in NPSLE, IL-1b and IL-1ra in MS, and IL-12(p70) and VEGF in NMO (Suppl. Fig. 2). Examinations of each single marker may be meaningless, because each marker operates together with one or more other markers in a rather complex system. These results indicate that the downstream pathway of Th1/Th2/Th17-related mediators differs among NPSLE, MS and NMO.

Various markers are known to be involved in the pathology of NPSLE; however, no predictive markers for detecting NPSLE were reported prior to the present study. Here, we were able to identify six minimum predictive markers (IL-17, IL-2, IFN-γ, IL-5, FGF-basic and IL-15), by using a weighted-voting algorithm with the highest MCC that could predict NPSLE from other autoimmune neurological diseases with 94.59% accuracy. We examined the correlations of cytokines/chemokines/growth factors in NPSLE, MS and NMO, and we found that many

cytokines/chemokines/growth factors were operating together in NPSLE compared to MS and NMO. The determination of various types of CSF cytokine profiles may contribute to the diagnosis of NPSLE and may help elucidate the mechanisms underlying this disease.

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Conflict of interest

The authors have no conflicts of interest to report.

Figure legends

Supplementary Fig. 1. Representative expressions of IL-17, IL-2, IFN-γ, IL-5, FGF-basic and IL-15 in the patients with NPH, VM, NPSLE, MS or NMO.

Supplementary Fig. 2. Heat-maps of nearest-neighbor correlations of cytokines, chemokines and growth factors in patients with NPSLE, MS and NMO. Among the cytokines, chemokines and growth factors analyzed, the distances of each pair of them were based on Spearman's correlation coefficient.

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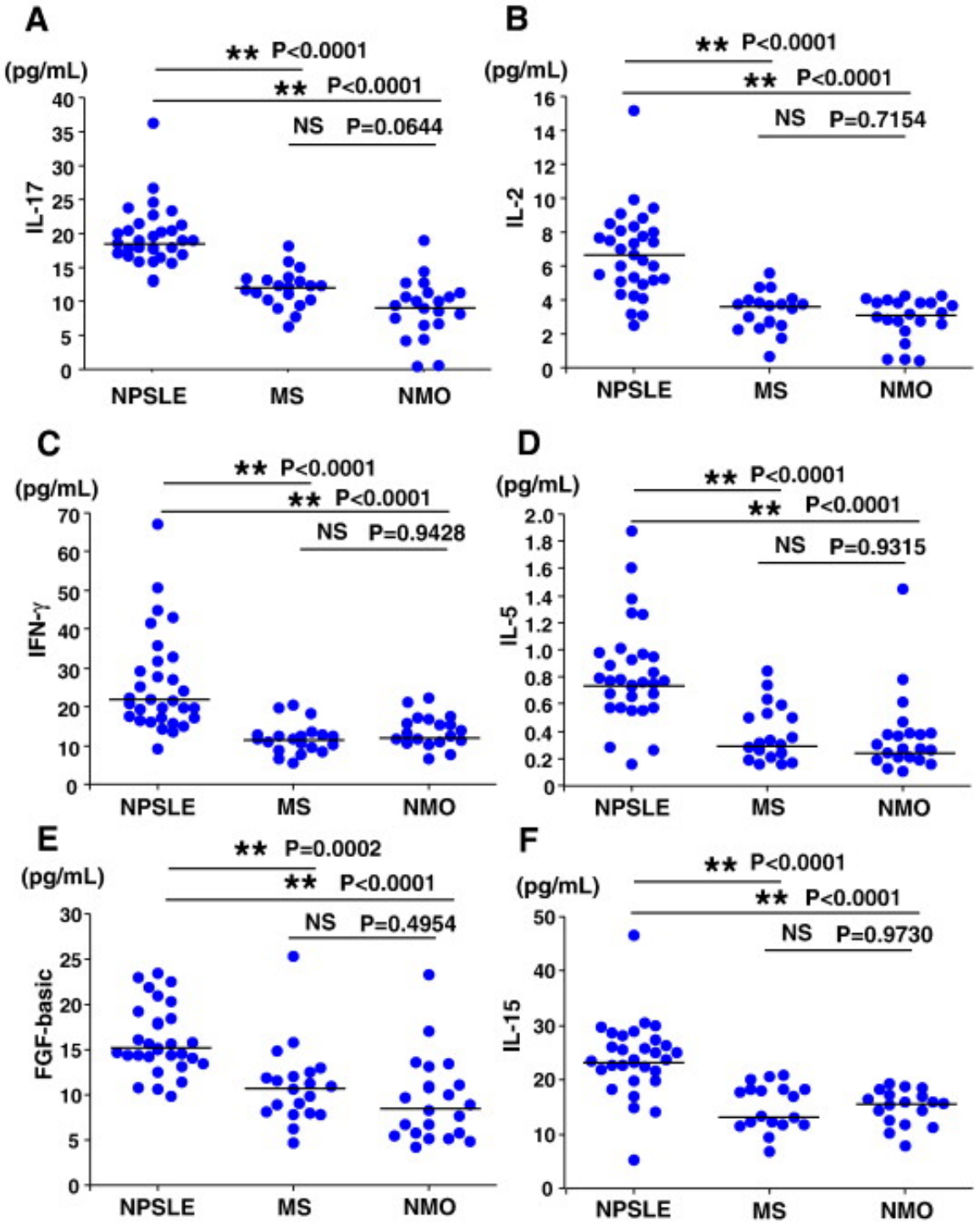
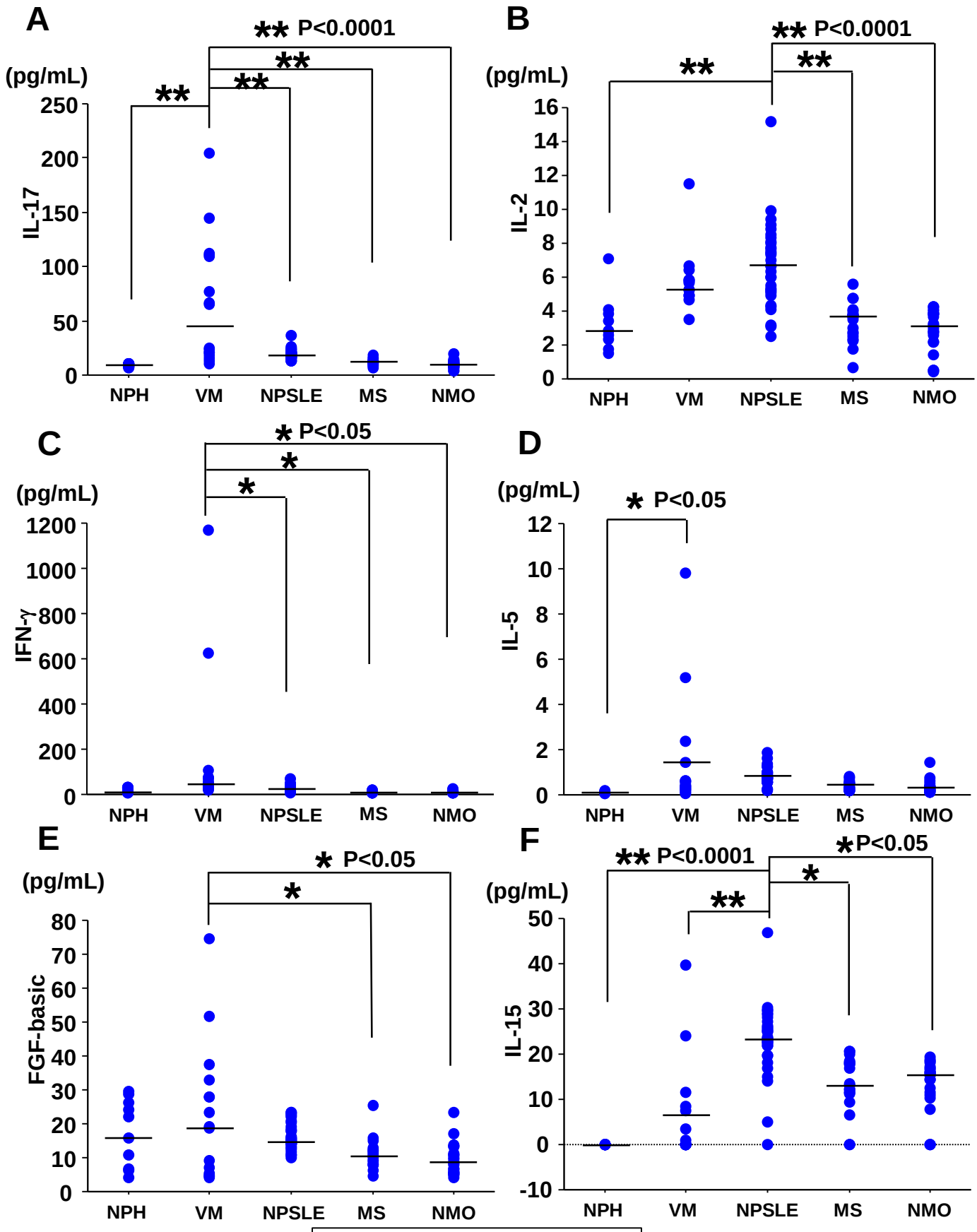
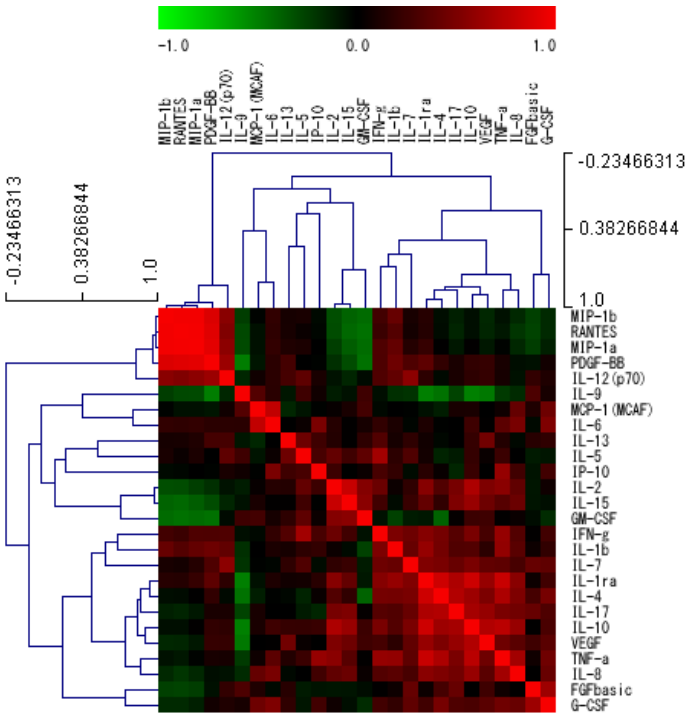

A
(pg/mL)
IL-17
** P<0.0001
** P<0.0001
NS P=0.0644
NPSLE
MS
NMO
B
(pg/mL)
IL-2
** P<0.0001
** P<0.0001
NS P=0.7154
NPSLE
MS
NMO
C
(pg/mL)
IFN-γ
** P<0.0001
** P<0.0001
NS P=0.9428
NPSLE
MS
NMO
D
(pg/mL)
IL-5
** P<0.0001
** P<0.0001
NS P=0.9315
NPSLE
MS
NMO
E
(pg/mL)
FGF-basic
** P=0.0002
** P<0.0001
NS P=0.4954
NPSLE
MS
NMO
F
(pg/mL)
IL-15
** P<0.0001
** P<0.0001
NS P=0.9730
NPSLE
MS
NMO

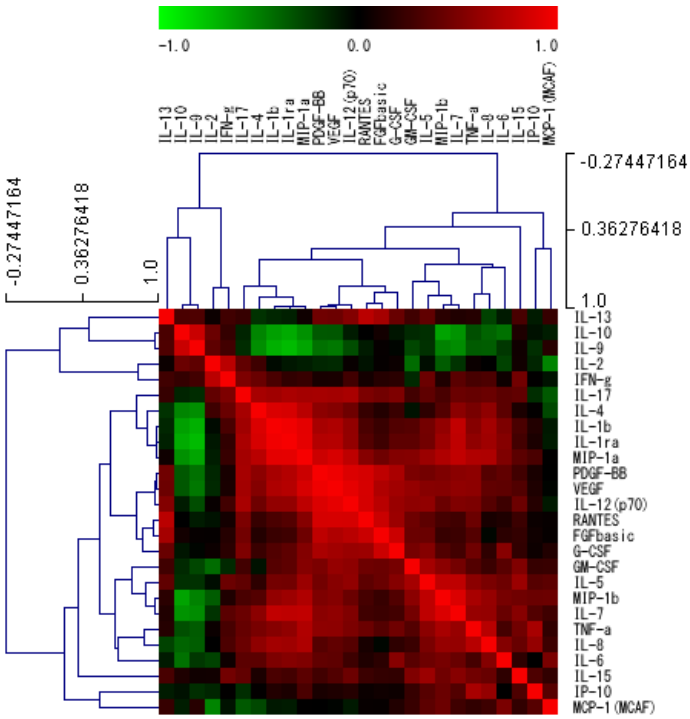
Figure 1


A
** P<0.0001
**
**
**
(pg/mL)
IL-17
250
200
150
100
50
0
NPH
VM
NPSLE
MS
NMO
B
** P<0.0001
**
**
(pg/mL)
IL-2
16
14
12
10
8
6
4
2
0
NPH
VM
NPSLE
MS
NMO
C
* P<0.05
*
*
(pg/mL)
IFN-γ
1200
1000
800
600
400
200
0
NPH
VM
NPSLE
MS
NMO
D
* P<0.05
(pg/mL)
IL-5
12
10
8
6
4
2
0
NPH
VM
NPSLE
MS
NMO
E
* P<0.05
*
(pg/mL)
FGF-basic
80
70
60
50
40
30
20
10
0
NPH
VM
NPSLE
MS
NMO
F
** P<0.0001
*P<0.05
*
**
(pg/mL)
IL-15
50
40
30
20
10
0
-10
NPH
VM
NPSLE
MS
NMO

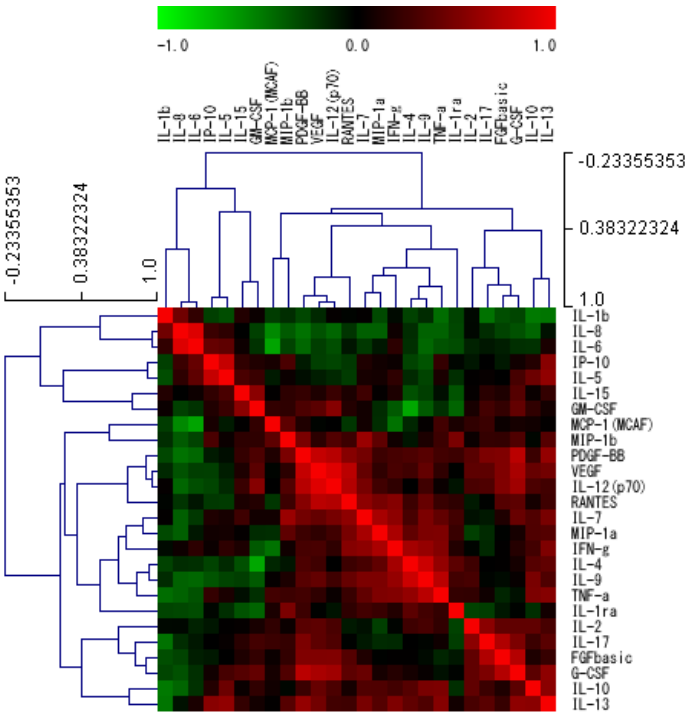
Suppl. Fig.1



NPSLE



MS



NMO

Suppl. Fig.2

Table 1. Comparison of each marker in cerebrospinal fluid among NPSLE, MS and NMO. *P*-value of each disease vs. the other two is shown.

Marker	NPSLE vs. Others	MS vs. Others	NMO vs. Others	Up/down of NPSLE vs. Others
IL-17	7.1981E-13	0.005200983	6.06716E-08	up
IL-2	1.79273E-09	0.000183297	3.05064E-07	up
FGF-basic	3.17974E-08	0.048298753	0.000240424	up
IL-5	1.73358E-07	0.001280693	0.000544469	up
IL-15	2.32436E-07	0.003814834	0.000574384	up
IL-9	3.0018E-07	0.000287076	0.041266563	up
IFN-γ	1.15444E-06	1.34983E-05	0.000209175	up
IL-12(p70)	2.06811E-06	0.016465613	0.001083612	up
IL-10	1.28056E-05	0.001591964	0.00011084	up
IL-7	2.57896E-05	0.01531653	0.004787909	up
GM-CSF	0.000174931	0.822364295	4.22879E-05	up
IL-13	0.000194842	0.023994687	0.022546951	up
TNF-α	0.000984547	0.002751192	0.012722463	up
Eotaxin	0.002694469	0.005361471	0.00337151	up
IL-8	0.923993657	0.003915445	0.106276224	up
IL-6	0.974937316	0.014641205	0.153928475	up

Table 2. Ranking of the cytokines/chemokines for the diagnosis of NPSLE determined by a weighted-voting algorithm with the highest Matthews correlation coefficient

Marker	TP	FN	TN	FP	Sensitivity	Specificity	Accuracy	Positive Predictive Value	Negative Predictive Value	MCC
IL-17	29	3	38	4	90.63%	90.48%	90.54%	87.88%	92.68%	80.83%
IL-2	28	4	39	3	87.50%	92.86%	90.54%	90.32%	90.70%	80.69%
IFN-γ	28	4	41	1	87.50%	97.62%	93.24%	96.55%	91.11%	86.38%
IL-5	28	4	41	1	87.50%	97.62%	93.24%	96.55%	91.11%	86.38%
FGF-basic	28	4	40	2	87.50%	95.24%	91.89%	93.33%	90.91%	83.49%
IL-15	30	2	40	2	93.75%	95.24%	94.59%	93.75%	95.24%	88.99%
IL-8	7	25	39	3	21.88%	92.86%	62.16%	70.00%	60.94%	21.35%
IL-6	7	25	39	3	21.88%	92.86%	62.16%	70.00%	60.94%	21.35%

TP: true positives (predicted positive, actual positive); FN: false negatives (predicted negative, actual positive); TN: true negatives (predicted negative, actual negative); FP: false positives (predicted positive,actual negative). MCC: Matthews correlation coefficient.

Supplementary Table 1. Baseline variables of the 32 neuropsychiatric systemic lupus erythematosus (NPSLE) patients

Gender (female %)	96.9%
Age, median (range), yrs	34.9 (15–50)
SLE duration, median (range), yrs	8.7 (0.1–33)
SELENA-SLEDAI score, median (range)	13.3 (2–28)
anti-ds DNA antibodies, median (range), U/mL	20.2 (0.4–274)
C3, median (range),mg/dL	86.3 (31.1–143)
C4, median (range), mg/dL	19.4 (2.1–50.3)
CSF IgG index	0.61 (0.47–0.87)
% of APS patients	7/32 (21.9%)
% of anti-ribosomal P antibody	8/32 (25.0%)
% of abnormal MRI findings	17/32 (53.1%)

SELENA-SLEDAI: The NPSLE patients' Safety of Estrogens in Lupus Erythematosus National Assessment–Systemic Lupus Erythematosus Disease Activity Index, CSF: cerebrospinal fluid, APS: antiphospholipid syndrome.

Supplementary Table 2. Neuropsychiatric manifestations of the 32 NPSLE patients

Central nervous system	
Headache, n (%)	14 (35.9)*
Psychosis, n (%)	10 (25.6)*
Mood disorder, n (%)	5 (12.8)*
Cognitive dysfunction, n (%)	2 (5.1)
Acute confusional state, n (%)	2 (5.1)*
Cerebrovascular disease, n (%)	2 (5.1)*
Seizure disorders, n (%)	2 (5.1)*
Cranial neuropathy, n (%)	1 (2.6)*
Peripheral nervous system	
Mononeuropathy, single/multiplex, n (%)	1 (2.6)*

*including patients who had more than one manifestation.

Supplementary Table 3. Formulas for determining the prediction strength value using six markers

Marker	Formula of weight vote value
IL-17	$V_{IL\text{-}17} = [X_a - (19.33+10.31)/2] \times (-1.07)$
IL-2	$V_{IL\text{-}2} = [X_a - (6.70+3.10)/2] \times (-0.99)$
IFN-γ	$V_{IFN\text{-}\gamma} = [X_a - (25.73+12.29)/2] \times (-0.80)$
IL-5	$V_{IL\text{-}5} = [X_a - (0.82+0.37)/2] \times (-0.73)$
FGF-basic	$V_{FGF\text{-}basic} = [X_a - (16.24+10.19)/2] \times (-0.72)$
IL-15	$V_{IL\text{-}15} = [X_a - (23.12+13.25)/2] \times (-0.71)$
PS value	$\frac{V_{IL\text{-}17}+V_{IL\text{-}2}+V_{IFN\text{-}\gamma}+V_{IL\text{-}5}+V_{FGF\text{-}basic}+V_{IL\text{-}15}}{\lvert V_{IL\text{-}17}+V_{IL\text{-}2}+V_{IFN\text{-}\gamma}+V_{IL\text{-}5}+V_{FGF\text{-}basic}+V_{IL\text{-}15} \rvert}$
Criterion	Positive….others Negative…. NPSLE

The weighted vote value (v_a) of each of the six markers was obtained by the formulas shown. X_a is the expression level of each marker 'a' in a CSF sample.

Supplementary Table 4. Comparison of each marker in serum among NPSLE and other autoimmune neurological diseases (non-NPSLE). P-value of NPSLE vs. non-NPSLE

Marker	NPSLE	non-NPSLE	p-value
PDGF-BB	3019±326	3507±334	0.3015
IL-1β	1.72±0.41	2.50±0.42	0.2035
IL-1ra	93.5±38.3	202.1±39.2	0.0672
IL-2	1.46±0.83	3.56±0.85	0.0963
IL-4	4.66±0.42	5.91±0.43	0.0515
IL-5	0.56±0.06	0.66±0.06	0.3030
IL-6	15.4±3.19	20.5±3.27	0.2659
IL-7	8.38±1.02	8.32±1.04	0.9694
IL-8	43.6±17.8	31.4±18.3	0.6296
IL-9	154.6±34.8	156.8±35.7	0.9647
IL-10	2.94±0.58	4.65±0.59	0.0511
IL-12(p70)	12.4±1.82	16.0±1.87	0.1811
IL-13	8.36±2.23	13.2±2.29	0.1493
IL-15	0.00±0.89	3.13±0.92	0.0275*
IL-17	55.8±5.30	61.8±5.43	0.4374
Eotain	29.4±5.00	44.0±5.12	0.0547
FGF-basic	8.26±1.02	9.73±1.05	0.3250
G-CSF	6.83±0.76	8.33±0.81	0.2040
GM-CSF	31.4±7.53	14.3±7.72	0.1180
IFN-γ	41.5±5.57	62.5±5.70	0.0164*
IP-10	979.7±198.3	612.5±203.2	0.2017
MCP-1	36.5±6.28	26.9±6.43	0.2891
MIP-1a	7.09±1.51	3.76±1.55	0.1306
MIP-1b	90.9±10.5	87.6±10.8	0.8247
RANTES	8076±2008	9526±2058	0.6225
TNF-α	27.6±4.56	40.7±4.67	0.0618
VEGF	164.2±29.8	228.2±30.5	0.1484

*Significant differences between the groups ($p<0.05$)