Clinical Immunology 237 (2022) 108980

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Measurement of anti-suprabasin antibodies, multiple cytokines and chemokines as potential predictive biomarkers for neuropsychiatric systemic lupus erythematosus

Trang T.T. Hoang [a,1], Kunihiro Ichinose [a,*,1], Shimpei Morimoto [b], Kaori Furukawa [a], Ly H.T. Le [c], Atsushi Kawakami [a]

[a] Department of Immunology and Rheumatology, Division of Advanced Preventive Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[b] Clinical Research Center, Nagasaki University Hospital, Nagasaki, Japan
[c] Graduate School of Engineering, Nagasaki University, Japan

ARTICLE INFO

Keywords:
Suprabasin
Cytokines
Chemokines
NPSLE
Bayesian posterior predictive values
SOIL

ABSTRACT

Neuropsychiatric systemic lupus erythematosus (NPSLE) varies in presentation and is one of the leading causes of morbidity and mortality among patients with SLE. This study determined the most critical serum biomarkers for the development of NPSLE as they may have clinical utility prior to the onset of neuropsychiatric symptoms. We retrospectively analyzed 35 NPSLE patients, 34 SLE patients, 20 viral meningitis (VM) patients, and 16 relapsing-remitting multiple sclerosis (MS) patients. We measured anti-suprabasin antibodies concentrations in serum by using Luciferase immunoprecipitation system (LIPS) assay. The serum concentrations of cytokines/chemokines were measured by using multiplex bead-based assay. We found serum FGF-2 level was significantly higher in the NPSLE group compared to the SLE group and the healthy control group. The anti-suprabasin antibody relative concentration (SRC) has high positive predictive values for the development of NPSLE. The most essential biomarkers are VEGF, anti-suprabasin antibodies, sCD40L, IL-10, GRO, MDC, IL-8, IL-9, TNF-α, MIP-1α.

1. Introduction

Systemic lupus erythematosus (SLE) is a life-long condition, the pathogenesis of which is believed to be loss of immunological tolerance and sustained autoantibody production [1]. Patients with SLE can present with various manifestations involving multiple organ damage. Neuropsychiatric SLE (NPSLE) varies in presentation and is one of the leading causes of morbidity and mortality among patients with SLE. NPSLE can occur at an early stage of SLE and manifests in 39% - 50% of SLE patients [2]. Distinguishing NPSLE from other neuropsychiatric conditions with different etiologies is challenging. The pathogenesis of NPSLE is multifactorial and involves diverse cytokines, autoantibodies and immune complexes inducing blood-brain barrier (BBB) dysfunction, neuroendocrine-immune imbalance, vascular occlusion, tissue, and neuronal damage.

Several pro-inflammatory cytokines such as B cell-activating factor (BAFF), tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK), interferon (IFN)-α, IFN-γ, interleukin (IL)-2, IL-6, IL-8, IL-10 have been implicated in the pathogenesis of NPSLE [3]. The increased serum levels of IL-6, IL-10, macrophage-derived chemokine (MDC), and vascular endothelial growth factor (VEGF) have been reported in SLE and were correlated to the disease activity [4–6]. In cerebrospinal fluid (CSF), IL-6 is a major pro-inflammatory cytokine associated with psychiatric NPSLE and may be produced by neurons, endothelial cells and glial cells [7]. In a previous study we found no significant difference in the CSF IL-6 levels among NPSLE, MS and NMO patients [8]. Another study found that concentrations of IL-6, IL-8, IP-10, MCP-1 and G-CSF in the CSF of NPSLE patients are higher than in non-NPSLE patients, but not in sera [9]. The heterogeneous cytokine profiles in both serum and CSF reflect the diversity of clinical manifestations and pathogenic pathways in NPSLE. Therefore, identification of the biomarkers which are integral to the pathogenesis of the disease and correlate closely with

* Corresponding author at: Department of Immunology and Rheumatology, Division of Advanced Preventive Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.
E-mail address: kichinos@nagasaki-u.ac.jp (K. Ichinose).
[1] Dr. Trang Hoang and Dr. Kunihiro Ichinose contributed equally to this work.

https://doi.org/10.1016/j.clim.2022.108980
Received 8 January 2022; Received in revised form 8 March 2022; Accepted 9 March 2022
Available online 13 March 2022

disease activity and prognosis has gained considerable interest.

It has been postulated that the BBB disruption or leakage results in the access of pro-inflammatory cytokines/chemokines and autoantibodies to brain tissue [10]. In NPSLE, autoantibodies are passively transferred from circulation to the central nervous system (CNS) through dysfunctional BBB or produced intrathecally. In particular, anti-ribosomal P antibodies may induce neuronal apoptosis in cultured brain cells and living rat models [11]. Several studies have demonstrated a correlation between anti-ribosomal P antibodies with depression and psychosis in NPSLE [12]. Anti-neuronal antibodies mediate diffuse neuronal damage and debilitate synaptic transmission. Anti-N-methyl-D-aspartate receptor 2 (NR2) antibodies are anti-dsDNA antibodies that activate glutamate receptors and cause neuronal apoptosis. A recent study found the absence of anti-NR2 antibodies in both plasma and CSF of NPSLE patients, prompting a search for novel specific autoantibodies for NPSLE [13].

The suprabasin (SBSN) was initially found highly expressed in the suprabasal layers of the neonatal epidermis and was described as having a potential role in epidermal stratification [14]. SBSN was found upregulated in tumor endothelial cells compared to normal endothelial cells, and SBSN knockdown impedes migration and tube formation ability of tumor endothelial cells [15]. Previously we have shown that the titer of anti-SBSN antibodies in CSF of NPSLE patients was significantly higher than in SLE, MS and NPH groups [16]. An *in vitro* study indicated that anti-SBSN antibodies in CSF bound directly to the astrocytes and activated the senescence and autophagy pathways [16].

This study evaluated serum cytokines, chemokines, and anti-SBSN antibodies in NPSLE compared to SLE without neuropsychiatric symptoms, VM, MS and healthy control (HC) as disease controls. This analysis aimed to elucidate the predictive values of serum anti-SBSN antibodies and cytokines/chemokines for the development of NPSLE as this may have clinical utility prior to the onset of neuropsychiatric symptoms.

2. Study design and methods

2.1. Study design

2.1.1. Study population

The study population was 35 patients admitted to Nagasaki University Hospital throughout 2014 to 2020 and were diagnosed with SLE based on the revised criteria of the American College of Rheumatology for the classification of SLE [17]. Neuropsychiatric symptoms in SLE (NPSLE) were classified according to the American College of Rheumatology nomenclature and case definitions for NPSLE [18].

As disease controls, we used samples from 34 SLE patients without neuropsychiatric manifestations (the SLE group), 20 viral meningitis (VM) patients, and 16 relapsing-remitting multiple sclerosis (MS) patients from the Department Immunology and Rheumatology, Nagasaki University Hospital. The serum of 38 healthy people (HC) was used as the non-autoimmune, non-inflammatory control.

We measured anti-SBSN antibodies in serum using the luciferase immunoprecipitation system (LIPS) assay described in the methods session. We also measured 38 multiple cytokines and chemokines in sera of NPSLE and SLE patients to identify the potential correlation between anti-SBSN antibodies and cytokines/chemokines. We used the combined dataset of anti-SBSN antibodies and cytokines/chemokines to identify the most critical biomarkers in the prediction of diagnosis of NPSLE. Some of the patients provided written informed consent for the use of their data and sample and the opt-out strategy was used by the remainder of the patients. Patients who declined to give informed consent were excluded. The protocol was approved by the Nagasaki University Hospital Clinical Research Ethics Committee (approval#17082129).

2.1.2. Medical record review of patients' profiles

Patients' medical records were retrospectively reviewed. Demographic information including age and gender, neuropsychiatric symptoms, and serum laboratory results on admission in NPSLE and SLE patients were extracted from medical records. The serum autoantibodies of anti-double-stranded DNA (dsDNA), anti-Smith (Sm), anti-ribosomal P antibodies, and antiphospholipid antibodies were collected. The anti-dsDNA antibodies, anti-Smith antibodies, and anti-ribosomal P antibodies were determined by enzyme-linked immunosorbent assay (ELISA). The antiphospholipid antibodies result was positive when either anticardiolipin IgG or IgM and anticardiolipin-β2-glycoprotein I complex antibodies using a commercial ELISA or lupus anticoagulant by the dilute Russell's Viper Venom Time (dRVVT) method was detected in serum. The diagnostic criteria of antiphospholipid syndrome (APS) were based on a previous report [19]. The CSF IL-6 level, and CSF IgG index were measured. We evaluated the patients' SLE disease activity on admission using the Safety of Estrogens in Lupus Erythematosus National Assessment–Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) [20]. The diagnosis of lupus nephritis (LN) was based on renal biopsy-proven 2003 International Society of Nephrology/Renal Pathology Society (ISN/RPS) LN classification [21].

2.2. Methods

2.2.1. Luciferase immunoprecipitation system (LIPS) assay for the detection of autoantibodies to SBSN

To generate luciferase reporters for the human SBSN, we fused full-length human SBSN cDNA (NM_001166034; GenScript, Piscataway, NJ) to NanoLuc (Nluc) luciferase. Human embryonic kidney (HEK) 293F cells (Thermo Fisher Scientific) were then transfected with the expression plasmid encoding SBSN-NLuc with FuGENE6 (Promega). Two days later, the supernatant of the transfected cells was collected and used as the SBSN-NLuc reporter solution. This protocol was similar to the process of generation of Gaussia luciferase reporter as described [16].

To achieve serum dilution, 15 μL serum was diluted with 485 μL phosphate-buffered saline (PBS). To purify IgG antibodies in the diluted serum, we used spin columns type IgG antibody-purify kit consisting of immobilized protein G (Cosmo Bio Co., LTD, Tokyo, Japan) on the solid phase surface of filter-type monolithic silica with continuous pores.

To confirm the accuracy of the LIPS assay and quantify the anti-SBSN antibodies, we used a commercially available antibody to human SBSN (NBP2–49528, Novus Biologicals, USA) as standard controls. The NBP2–49528 anti-SBSN antibody was diluted with distilled water into three concentrations 0.1 ng/μL, 0.01 ng/μL and 0.005 ng/μL. To detect anti-SBSN antibodies, 40 μL of the SBSN-NLuc reporter solution was mixed with 550 μL of diluted serum after the purification step or 150 μL diluted NBP2–49528 anti-SBSN antibody samples. PBS with 3% bovine serum albumin and 0.05% Tween®20 was then added into sera and standard control mixed solutions, 200 μL and 600 μL, respectively. The final mixed solutions were incubated overnight at 4 °C with rotating.

The mixed solution was then incubated with 25 μL of protein G Mag Sepharose (GE Healthcare, Little Chalfont, Buckinghamshire, UK) with end-to-end mixing at room temperature in 30 min. Following centrifugation and three washes with PBS containing 0.05% Tween®20, the bioluminescence activities of the luciferase reporters in protein G Mag Sepharose were measured with a Nano-Glo® Luciferase Assay kit (Promega) and a GLOMAX 20/20 Luminometer (Promega). The luminometer output was measured in relative luminescence units (RLUs).

The antibody levels were expressed as anti-SBSN antibodies relative concentrations (SRC), which were calculated as follows:

SRC = [measurement value of the sample serum (RLU)]/[measurement value of the control sample with 0.005 ng/μL NBP2–49528 SBSN antibody (RLU)]

2.2.2. Multiplex cytokines and chemokines magnetic bead assay

Serum of NPSLE and SLE patients were analyzed using Milliplex MAP Human cytokines/chemokines premixed 38 plex kit (EMD Millipore Corp, Billerica, MA, USA) on the MAGPIX® with xPONENT® software (Luminex Corporation, Austin, TX, USA). The assay procedure was performed according to the manufacturer's instructions. The level of 38 cytokines and chemokines including epidermal growth factor (EGF), CCL11/eotaxin, basic fibroblast growth factor (FGF-2/bFGF), FMS-like tyrosine kinase 3 ligand (FLT-3 L), fractalkine, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), chemokine (C-X-C motif) ligand 1 (CXCL1/GRO), IFN-γ, IFN-α2, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IL-1α,IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, C-X-C motif chemokine ligand 10 (CXCL10/IP-10), monocyte chemoattractant protein-1 (MCP-1/CCL2), MCP-3, MDC, CCL3/macrophage inflammatory protein (MIP)-1α, CCL4/MIP-1β, transforming growth factor (TGF)-α, TNF-α, TNF-β, VEGF, and soluble CD40 ligand (sCD40L) were measured. A brief protocol explanation was mentioned [22]. The Median Fluorescent Intensity (MFI) data uses a 5-parameter logistic or spline curve-fitting method for calculating cytokines/chemokines concentrations in samples. Minimum detectable concentration (MinDC) was calculated using MILLIPLEX® analyte 5.1. The ranges of each cytokine/chemokine in Quality Control 1 and 2 are located on the EMD Millipore website (emdmillipore.com).

2.3. Statistical analysis

The Kruskal-Wallis test was used for intergroup comparisons of continuous non-normal distributed variables. The chi-squared test was used to compare the distributions of categorical variables between groups. The Conover-Iman *post hoc* test was used for multiple pairwise comparisons with one control group for continuous non-normal distributed variables, *p*-values were adjusted by using Holm's method.

We used robust linear regression to infer mean differences of SRC value between the NPSLE and each of the other disease groups [23].

The cut-off value of SRC and its predictive values for the prediction of NPSLE were determined using a Bayesian model described by Vradi et al. [24]. The binary response variable Y took the value 1 when a patient was diagnosed with NPSLE and 0 when a patient was diagnosed with SLE. X was the continuous measurement of the SRC in NPSLE and SLE patients. In this study, the positive predictive value (PPV) of the cut-off value (cp) was expected to be ≥70%, while the negative predictive value (NPV) was ≥50%. The Bayesian analysis to compute cut-off value was performed in SAS Studio (SAS Institute Inc., 2015).

To identify the most critical biomarkers in the prediction of NPSLE, we analyzed anti-SBSN antibodies SRC and levels of 38 cytokines/chemokines in the serum of NPSLE and SLE patients. We used the sparsity-oriented important learning (SOIL) procedure which was proposed by C. Ye et al. to measure the variable importance of the binary classification model [25]. The SOIL importance was measured by using an R package SOIL [26].

All of the statistical analyses were done in R version 4.1.0 [27] . The significance level was set at $p < 0.05$.

3. Results

Baseline characteristics of patients included in this study are shown in Table 1**.** The neuropsychiatric symptoms were classified according to the American College of Rheumatology nomenclature and case definitions for NPSLE and were summarized in **Suppl. Table S1.**

3.1. The LIPS assay detected serum antibodies against SBSN in NPSLE patients

The anti-SBSN antibodies were detected in the serum of all NPSLE, SLE, VM, and MS patients. The median [IQR] of SRC in the NPSLE, SLE, VM and MS groups were 2.70 [2.10; 3.37], 2.23 [1.72; 2.93], 2.06[1.51; 2.47] and 2.20[0.86; 2.58], respectively (Fig. 1). We found that the SRC of anti-SBSN antibodies was significantly higher in the NPSLE group than VM and MS groups ($p = 0.011$ and $p = 0.039$, respectively). We did

Table 1
Baseline characteristics.

	NPSLE	SLE	VM	MS	*p*-value
	N = 35	*N = 34*	*N = 20*	*N = 16*	
Age, year	43.0 [29.0;50.0]	33.5 [29.5;46.8]	55.0 [30.0;70.0]	42.5 [34.5;51.5]	
Gender: Female	25 (71.4%)	32 (94.1%)	6 (30.0%)	11 (68.8%)	
Disease duration,year	5.00 [1.50;15.0]	2.75 [0.10;10.0]	–	–	0.155[a]
SELENA-SLEDAI, score	12.0 [8.00;16.0]	9.50 [6.00;12.0]	–	–	0.516[a]
anti-dsDNA antibodies(U/ml)	5.50 [2.60;26.6]	13.2 [6.00;38.8]	–	–	0.032[a]
anti-Sm antibodies (U/ml)	1.85 [1.00;13.7]	6.70 [1.60;45.2]	–	–	0.205[a]
APS, positive	15 (42.9%)	5 (14.7%)	–	–	0.021[b]
LN, positive	7 (20.0%)	17 (50.0%)	–	–	0.018[b]
CSF IL-6(pg/ml)	3.80 [2.30;10.8]	4.20 [2.20;8.10]	–	–	0.729[a]
C3 (mg/dl)	70.8 [60.7;90.4]	64.0 [48.0;74.2]	–	–	0.037[a]
C4(mg/dl)	15.9 [10.2;20.4]	9.65 [6.55;18.3]	–	–	0.063[a]
Anti-ribosomal P antibodies(index):					0.123[b]
Negative	21 (60.0%)	14 (41.2%)	–	–	
Positive	5 (14.3%)	3 (8.82%)	–	–	
Information not available	9 (25.7%)	17 (50.0%)	–	–	
IgG index	0.54 [0.46;0.61]	0.51 [0.47;0.59]	–	–	0.652[a]

Data is presented by median [IQR] and percentage.
[a] *p*- values were determined by Kruskal-Wallis test.
[b] *p*- values were determined by Chi-squared test .

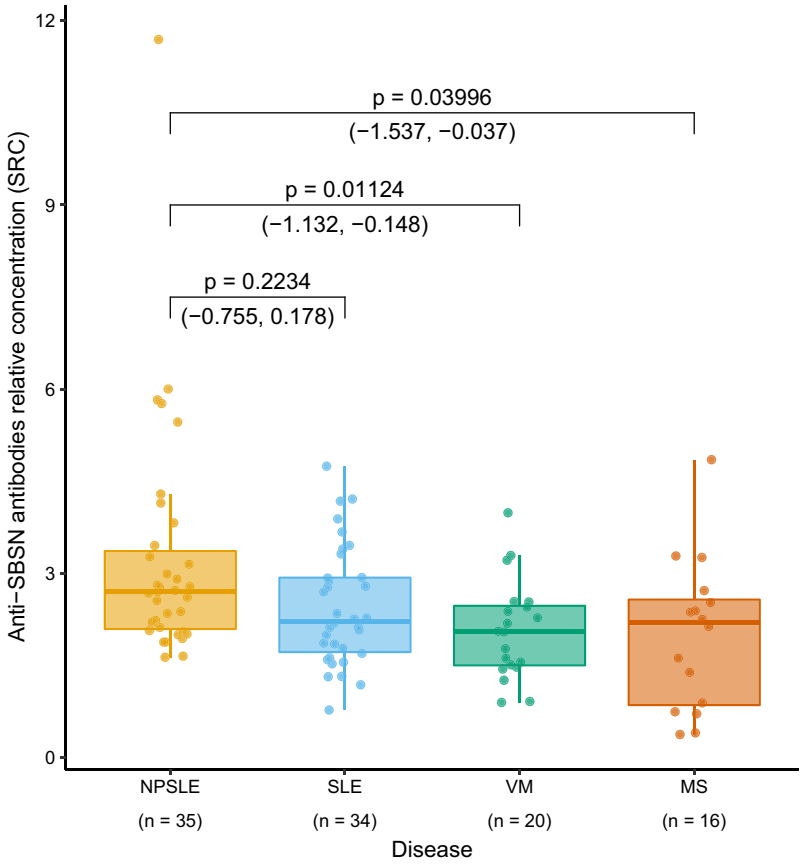

Fig. 1. The LIPS assay for anti-SBSN antibodies in sera from the patients with NPSLE, SLE, VM and MS.

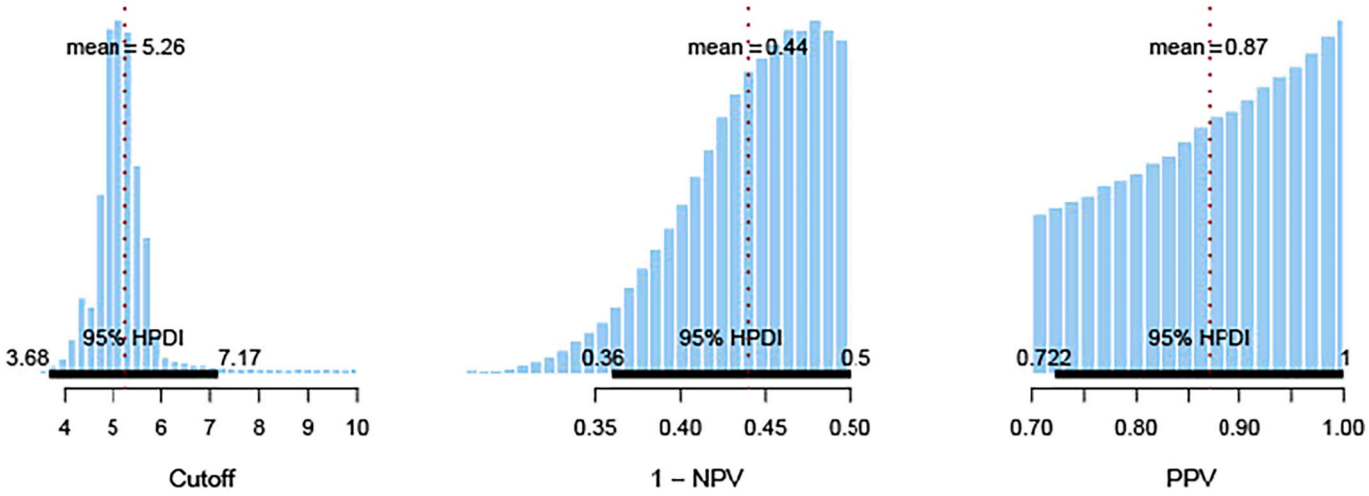

Fig. 2. Summary of the posterior distribution of the cutoff of SRC and its predictive value (1-NPV and PPV). The vertical red dotted lines indicate the mean of the distribution. The 95% HPDI is shown as the thick black horizontal line with the boundaries written above the line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

not find a significant difference in the means of SRC between the NPSLE group and the SLE group ($p = 0.22$).

There was no significant difference in the means of SRC between the NPSLE group and the HC group ($p = 0.185$) (**Suppl. Fig. S1**).

3.2. Bayesian method measured the cut-off and predictive values of the relative concentrations of anti-SBSN antibodies for the prediction of NPSLE

To evaluate the predictive values of anti-SBSN antibodies, we applied the Bayesian method proposed by Vradi et al. to estimate the cut-off of SRC-related to NPSLE diagnosis. First, we checked the quality of finite Markov chain Monte Carlo (MCMC) to determine whether there were

Table 2
Comparison of each cytokines/chemokines in serum of NPSLE, SLE patients and HC.

	HC ($N = 38$)	NPSLE ($N = 35$)	SLE ($N = 34$)	P Overall		HC (N = 38)	NPSLE (N = 35)	SLE (N = 34)	P Overall
EGF	85.9 [47.5;134]	82.5 [42.0;146]	69.6 [43.3;95.1]	0.519	IL-8	4.42 [2.59;6.38]	23.9 [9.52;60.7]	23.9 [8.98;34.6]	<0.001
EOTA	85.2 [72.8;106]	147 [105;174]	114 [75.1;190]	0.001	IL-9	0.00 [0.00;0.00]	0.00 [0.00;5.46]	0.00 [0.00;2.32]	0.007
FGF2	22.1 [14.9;39.4]	57.9 [38.0;82.6]	43.0 [27.2;69.0]	<0.001	IFN-α2	0.00 [0.00;0.00]	23.2 [2.00;66.6]	18.8 [0.00;31.2]	<0.001
FLT3L	0.00 [0.00;0.00]	0.00 [0.00;16.3]	0.00 [0.00;15.5]	0.084	IP-10	181 [160;240]	957 [507;2468]	984 [557;1699]	<0.001
FRAC	0.00 [0.00;12.1]	39.7 [14.8;129]	22.9 [16.7;39.5]	<0.001	MCP-1	382 [276;443]	662 [507;955]	536 [409;832]	<0.001
GCSF	0.00 [0.00;2.76]	28.4 [10.5;72.1]	14.6 [3.81;31.8]	<0.001	MCP-3	0.00 [0.00;13.2]	30.1 [8.94;64.1]	17.7 [0.00;64.5]	<0.001
GMCSF	1.28 [0.04;2.70]	7.16 [2.76;16.9]	4.64 [3.21;8.57]	<0.001	MDC	682 [474;774]	426 [270;700]	453 [328;842]	0.099
GRO	795 [555;993]	785 [510;1261]	868 [451;1365]	0.869	IL-13	0.00 [0.00;0.00]	0.00 [0.00;7.70]	0.00 [0.00;1.69]	0.005
IFN-γ	0.00 [0.00;1.82]	6.63 [3.16;19.1]	4.04 [1.12;9.59]	<0.001	IL-15	0.00 [0.00;1.10]	5.38 [2.59;8.46]	3.36 [2.17;7.15]	<0.001
IL-10	0.00 [0.00;0.00]	9.34 [2.46;18.2]	4.06 [1.20;12.7]	<0.001	IL-17	0.00 [0.00;0.00]	0.09 [0.00;4.00]	0.00 [0.00;0.53]	0.060
IL-12p40	0.00 [0.00;0.00]	0.00 [0.00;21.4]	0.00 [0.00;6.58]	<0.001	IL-1α	0.00 [0.00;0.00]	0.00 [0.00;9.98]	0.00 [0.00;6.24]	0.010
IL-12p70	0.00 [0.00;0.00]	1.10 [0.00;5.17]	0.00 [0.00;2.78]	<0.001	IL-1β	0.00 [0.00;0.00]	0.31 [0.00;3.00]	0.00 [0.00;0.95]	0.001
IL-3	0.10 [0.00;0.11]	0.12 [0.00;0.20]	0.10 [0.00;0.16]	0.254	IL-1Ra	0.00 [0.00;8.06]	31.5 [17.2;72.7]	17.5 [7.59;39.7]	<0.001
IL-4	0.00 [0.00;11.0]	8.97 [0.00;66.6]	4.58 [0.00;30.4]	0.071	IL-2	0.00 [0.00;0.00]	0.00 [0.00;1.12]	0.00 [0.00;0.14]	0.014
IL-5	0.21 [0.00;0.80]	1.12 [0.32;2.06]	0.71 [0.28;1.74]	<0.001	MIP-1α	0.00 [0.00;1.97]	5.72 [2.41;8.21]	3.86 [1.76;8.60]	<0.001
IL-6	0.00 [0.00;0.00]	0.00 [0.00;16.9]	0.00 [0.00;16.7]	0.002	MIP-1β	48.6 [29.5;67.7]	65.5 [48.5;94.6]	64.7 [35.0;86.0]	0.051
IL-7	0.00 [0.00;0.00]	4.70 [0.00;16.3]	2.78 [0.00;8.98]	<0.001	TGF-α	1.83 [1.39;2.34]	2.37 [1.41;3.64]	1.54 [1.02;2.78]	0.329
VEGF	73.5 [32.1;132]	120 [45.6;176]	50.8 [0.00;99.0]	0.029	TNF-α	7.81 [5.47;10.6]	23.7 [16.5;42.9]	30.2 [16.4;39.0]	<0.001
sCD40L	3780 [1916;5161]	1935 [1560;4467]	1744 [1252;3337]	0.006	TNF-β	0.00 [0.00;0.00]	0.00 [0.00;1.88]	0.00 [0.00;1.27]	0.095

All values are shown in median [IQR] with each unit is pg/ml.
EGF: epidermal growth factor, EOTA: eotaxin, FGF-2:fibroblast growth factor, FLT-3 L:FMS-like tyrosine kinase 3 ligand, fractalkine, G-CSF:granulocyte colony-stimulating factor, GM-CSF: granulocyte-macrophage colony-stimulating factor, CXCL1/GRO: chemokine (C-X-C motif) ligand 1, IFN-γ: interferon gamma, IL-1Ra: IL-1 receptor antagonist, CXCL10/IP-10: C-X-C motif chemokine ligand 10, MCP-1/CCL2: monocyte chemoattractant protein-1, MDC: macrophage-derived chemokine, MIP-1α/CCL3: macrophage inflammatory protein 1α, TGF-α: transforming growth factor alpha, TNF-α:tumor necrosis factor, VEGF: vascular endothelial growth factor, sCD40L: soluble CD40 ligand.
Multiple comparisons were performed by Kruskal-Wallis test (p overall).
The significance level was set at $p < 0.05$.

signs of unrepresentativeness or instability. The chains were converged and mixed well, which is a good sign that suggests representativeness of the posterior distribution. Sample autocorrelation of all parameters dropped quickly to zero with increasing lag. The Effective Sample Size (ESS) of all parameters was >17,500, and the Monte-Carlo Standard Error (MCSE) of all parameters was <0.0059. We confirmed that the MCMC chains were stable and accurate and were representative of the posterior distribution (**Suppl. Fig. S2**). The posterior distributions for the cut-off value of SRC and the predictive values 1-NPV and PPV are shown in Fig. 2. The Bayesian posterior mean of the cut-off of SRC was 5.26 with the 95% probability (highest posterior density interval (HPDI)) of the cut-off falls between the ranges from 3.68 to 7.17. The Bayesian posterior mean and 95% HPDI for PPV and 1-NPV were 0.87, (0.72; 1.0) and 0.44, (0.36; 0.5), respectively.

3.3. Comparison of cytokines/chemokines levels in serum of NPSLE, SLE patients and healthy controls

We analyzed the level of cytokines/chemokines in serum of 35 NPSLE patients, 34 SLE patients and 38 healthy people. We found that the level of FGF-2 in the serum of the NPSLE group is significantly higher than in the SLE group and the HC group (pairwise $p = 0.035$ and $p = 1.4 \times 10^{-8}$, respectively) (Table 2)**,** pairwise *p*-values were shown in **Suppl. Table S2**. The level of VEGF in serum of the NPSLE is significantly higher than in the SLE group but no different with the HC group (pairwise $p = 0.015$ and $p = 0.15$, respectively) (**Suppl. Table S2**). In the NPSLE group, the serum concentrations of EOTA, FRAC, G-CSF, GM-CSF, IFN-γ, IFN-α2, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IL-1α,IL-1β, IL-1, IL-1Ra, IL-2, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MCP-3, MDC, MIP-1α, MIP-1β, TNF-α are higher than in the HC group (pairwise p- values <0.05) **(Suppl. Table S2)**.

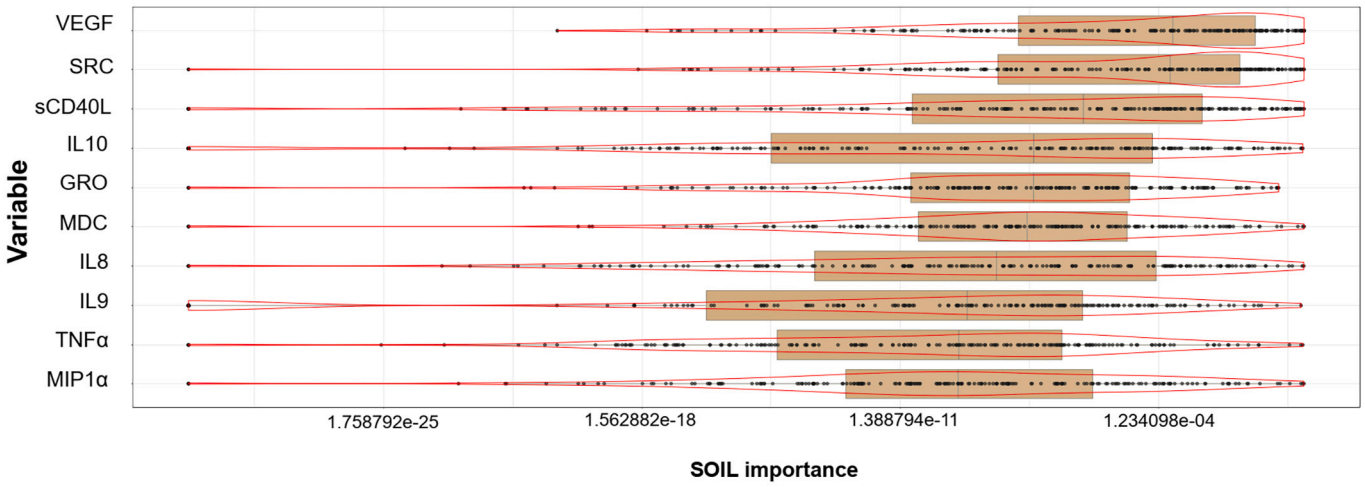

Fig. 3. Top 10 biomarkers having highest SOIL importance in prediction of NPSLE.

3.4. Variables' importance in a predictive model to diagnosis NPSLE

To list important variables that can be used to predict the occurrence of NPSLE, we measured SOIL importance of SRC and 38 cytokines/chemokines in the NPSLE and SLE groups. We identified the top 10 biomarkers in the variable importance as the components of the linear predictors for the diagnosis of NPSLE in the predictive model **(**Fig. 3**).** VEGF had the highest SOIL importance, followed by SRC, sCD40L, IL-10, GRO, MDC, IL-8, IL-9, TNF-α, MIP-1α. FGF-2 was ranked the 22nd important variable among 39 biomarkers (data not shown).

4. Discussion

In humans, SBSN is expressed in large amounts in stratified epithelia such as the skin, tongue, esophagus, forestomach, vagina, trachea, bladder and thymus [28]. In the last decade, SBSN has raised much research interest, especially in oncology, although the exact pathological function of SBSN currently remains unknown. The SBSN was upregulated in surviving cancer cells treated by 5-azacytidine, irradiation and IFN-γ, which implicates the SBSN gene is regulated by IFN-γ and other stress [29]. In the CSF, anti-SBSN antibodies index measured by LIPS assay was significantly higher in NPSLE compared to SLE, MS and normal pressure hydrocephalus (NPH) groups, but not in the serum corresponding to the CSF samples [16]. Of note, the CSF concentrations of IL-10, IL-13, GM-CSF, IP-10, and MCP-1 were significantly higher in the CSF anti-SBSN antibodies positive group of NPSLE patients than in the negative group [16]. These cytokines/chemokines are known to be upregulated in the CSF of NPSLE patients. In this study, we confirmed that although the mean of SBSN antibodies index in serum of the NPSLE group was higher than in the SLE group, there was no statistical difference ($p > 0.05$) (Fig. 1). We also found that serum anti-SBSN antibodies were not significantly different from healthy subjects. By using a flexible Bayesian method, we estimated the cut-off SRC associated with the targeted clinical utility by controlling the PPV of SRC to fall between 70% and 100% and 1-NPV to be between 0% to 50%. The 95% highest posterior density interval (HPDI) (also known as the 95% most credible value of the parameter) is the interval where any parameter value inside it has a higher probability density than any value outside [30]. As a result of our study, with 95% the cut-off SRC falls within 3.68 to 7.17, 95% probability of PPV values of the SRC is from 72% to 100%, and 95% probability of 1-NPV values of the SRC falls in the range from 36% to 50%. Our results provide convincing evidence that serum anti-SBSN antibodies is one of the predictive markers for the development of NPSLE.

We hypothesize that anti-SBSN antibodies in NPSLE may have different roles in serum than in the CSF. The presence of autoantibodies in the serum is not thought to reliably predict the development of NPSLE [31]. It has been suggested that additional 'hits' such as excessive stress or underlying infections may cause temporary destruction of the BBB, thereby promoting brain damage caused by serum-derived autoantibodies [32]. In the condition that the BBB is damaged, serum anti-SBSN antibodies may pass through the BBB to enter the brain and induce pathogenesis. The entry of anti-SBSN antibodies to the brain is one possible explanation for the indistinguishability in their serum concentrations between NPSLE and SLE groups. Additionally, leukocytes secrete pro-inflammatory cytokines infiltrate the central nervous system, promoting B cells survival and local antibodies production [32]. In other words, we believe that anti-SBSN antibodies in serum, not alone but in combination with multiple biomarkers, can be crucial in discriminating between NPSLE and SLE. Based on these results, the significance of anti-SBSN antibodies in serum and CSF as biomarkers is currently different and should be considered separately.

VEGF engages in various steps in angiogenesis including initial vasodilatation, increment of vascular permeability, reconstruction of the perivascular matrix and initiation of proliferation and migration of endothelial cells [33]. The permeability-enhancing activity of VEGF implies essential roles of this molecule in inflammation and other pathological circumstances [34]. Our present study found that VEGF levels in the serum of NPSLE groups were significantly higher than in the SLE group (**Suppl. Table S2**). Together with VEGF, FGF-2 involves the process of cellular migration, proliferation, and formation of new vessels [35]. FGF-2 was found to be significantly higher in serum of the SLE group than the control group, and serum VEGF level was positively correlated with FGF-2 level in the active SLE group [5]. We found serum FGF-2 level was significantly higher in the NPSLE group compared to the SLE group and the HC group (Table 2). In our study, the serum concentrations of EOTA, FRAC, G-CSF, GM-CSF, IFN-γ, IFNα2, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IL-1α,IL-1β, IL-1, IL-1Ra, IL-2, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MCP-3, MDC, MIP-1α, MIP-1β, TNF-α are higher in the NPSLE group than in the HC group. The IL-6 in serum were found elevated in patients with NPSLE and the highest was in an acute confusional state, the result suggested that serum IL-6 might play a role in the BBB breakdown NPSLE [36]. Interleukin-8 was suggested to alter the permeability of the BBB, attracts B cells and T cells to the site of inflammation. Neutrophils express receptors for CXC motif chemokine 1 (CXCR1) and CXC motif chemokine 2 (CXCR2), which bind to the CXCL1/GRO and CXCL8/IL-8. The abundance of IL-8 produced by tissue-resident macrophages and other cells at the site of infection leads to the migration of neutrophils. Lupus neutrophils can drive B and T cells abnormalities, enhancing the production of type 1 IFN, TNF-α, BAFF and APRIL [37]. According to a previous study, the serum levels of IL-6, IL-8, IL-1β and IFN-γ were found higher in the central nervous system neuropsychiatric systemic lupus erythematosus (CNS-NPSLE) than in the control group [38]. The study suggested the role of IFN-γ in inducing multiple ischemic foci [38]. In our previous study, CSF FGF-2 was among the six minimum predictive biomarkers for NPSLE [8]. The result implicates that there may be BBB dysfunction and increased vascular permeability enhanced by cytokines/chemokines in our NPSLE population study, leading to access to the brain of larger pathological molecules such as immune cells and autoantibodies.

Predictive biomarkers can be used to identify individuals who are prone to exposure to certain environmental factors or who have underlying predisposing health have conditions. The utility of predictive biomarkers is not confined to a clinical trial setting; but may also assist patient care in making decisions, such as determining treatment or selecting cost-effective interventions. To our understanding, this is the first time we have provided an overview of serum biomarkers that may be useful to predict NPSLE by providing a ranking of their importance. Our study population ranked 39 biomarkers based on their SOIL variable importance; methodology was implemented by Ye et al. [25]. Among these biomarkers, the ten highest SOIL variable importance biomarkers are VEGF, SRC, sCD40L, IL-10, GRO, MDC, IL-8, IL-9, TNF-α, MIP-1α, appeared in order (Fig. 3). Serum IL-10 concentration was found to be one of the indicators of depression in lupus patients, along with fatigue severity and relationship satisfaction [39]. Through inhibiting the expression of MIP-1α from monocytes and macrophages, IL-10 may indirectly regulate the effects of activated T lymphocytes [40]. MIP-1α participates in the acute inflammatory state through recruitment and activation of polymorphonuclear leukocytes. The levels of MIP-1α in active SLE patients was higher than in inactive SLE [41].

The majority of the production of sCD40L is from activated T lymphocytes, and activated platelets and sCD40L induce CD40-CD40 ligation with CD40 bearing cells [42]. An increased level of sCD40L has been found in SLE and other autoimmune diseases [42,43].

The serum MDC level was found to be significantly decreased in SLE patients compared to the control group, and may be a sensitive biomarker to determine disease progression or improvement [6]. TNF-α participates in the pathogenesis of depression through the activation of neuronal serotonin transporter causing serotonin uptake diminishment [44]. The serum TNF-α levels were increased in SLE patients with depressive symptoms and anxiety symptoms [44].

IL-9 is produced by Tregs, Th1, Th17 and the Th9 subset of T cells

under specific conditions. However, the precise role of IL-9 in the pathogenesis of SLE remains unknown. IL-9/IL-9R defect mitigate Experimental Autoimmune Encephalomyelitis (EAE) severity and enhance Tregs' activity, phenotypes reminiscent of SLE [45].

In our study population, we have 13/35 NPSLE patients who had more than one symptom (**Suppl. Table S1**). In order to investigate the relationship between specific neuropsychiatric (NP) symptoms and biomarkers, we select NPSLE patients who had only one symptom. In those with one NP symptom, there were 6 patients with headache, 6 patients with psychosis and 5 patients with cognitive dysfunction (data not shown). Since the sample sizes are small to perform predictive analysis, we present the mean comparisons between subgroups of NPSLE patients and SLE patients. There were no significant differences in the serum concentration of SRC and cytokines/chemokines between NPSLE headache and cognitive dysfunction subgroups and the SLE group (data not shown). In NPSLE psychosis patients, the levels of FGF2, FRAC, G-CSF, GM-CSF, IFN-γ, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-1α, IL-1β, IL-2, IL-1Ra, IFN-α2, MCP-3, TNF-α, TNF-β were significantly higher than in the SLE group ($p < 0.05$) (**Suppl. Fig. S3).** In patients with the first-episode psychosis, serum levels of IL-1α, IL-1β, IL-8 and TNF-α were higher compared to healthy controls [46]. None of the serum cytokines/chemokines that showed significant differences in our study were reported to be elevated in NPSLE psychosis, as far as we could determine.

One of the drawbacks of our study is that we were not able to produce a complete predictive model for NPSLE because we had available only small sample size. Instead, we chose to generate data to understand variable importance of the biomarkers to avoid the over-fitting model problem and to have reliable information that can be included in prediction of NPSLE. The variable volume gives researchers and clinicians a comprehensive understanding of the possible roles of serum biomarkers in predicting NPSLE other than trusting a single predictive model. Our findings are helpful for further research by providing a ranking of biomarkers that can be used as a reference for variable selection for the predictive model. In the clinical setting, they may assist clinicians to change or replace biomarkers for prediction and prognosis purposes in response to clinical considerations. On the other hand, the role of cytokines/chemokines and anti-SBSN antibodies appears to be inconsistent in relation to the heterogeneous manifestations of NPSLE. A larger patient group is required to establish a reliable predictive model for NPSLE, particularly in determining the relationship between specific clinical manifestations and biomarkers, and in term of predicting the progress of the disease.

5. Conclusions

In conclusion, our data demonstrated the ranking of serum biomarkers for the prediction of NPSLE. The most essential biomarkers are VEGF, anti-SBSN antibodies, sCD40L, IL-10, GRO, MDC, IL-8, IL-9, TNFα, MIP-1α. The cut-off value of SRC has high posterior positive predictive values for NPSLE. A larger study sample size is required to determine a predictive model for NPSLE, and in term of determining the relationship between specific clinical manifestations and biomarkers.

Ethics approval and consent to participate

This study was performed in accordance with the Declaration of Helsinki and was approved by the Nagasaki University Hospital Clinical Research Ethics Committee (approval no.17082129). Patients gave their informed consent to be subjected to the protocol.

Funding

This work was supported by the Japan Intractable Diseases Research Foundation (to K·I), the Nagao Memorial Foundation (to K·I) and Takeda Science Foundation (to K·I).

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgments

We wish to acknowledge Ms. Rika Hirayama for her technical support with preparing materials. We would like to thank Dr. Mark Bassett (Senior Medical Advisor, ISLHD NSW Health, Wollongong Australia) for his diligent proofreading of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2022.108980.

References

[1] G.C. Tsokos, M.S. Lo, P. Costa Reis, K.E. Sullivan, New insights into the immunopathogenesis of systemic lupus erythematosus, Nat. Rev. Rheumatol. 12 (12) (2016 Nov 22) 716–730.
[2] S. Sarwar, A.S. Mohamed, S. Rogers, S.T. Sarmast, S. Kataria, K.H. Mohamed, M. Z. Khalid, M.O. Saeeduddin, S.T. Shiza, S. Ahmad, et al., Neuropsychiatric systemic lupus erythematosus: a 2021 update on diagnosis, management, and current challenges, Cureus 13 (9) (2021 Sep 14), e17969.
[3] M. Govoni, J.G. Hanly, The management of neuropsychiatric lupus in the 21st century: still so many unmet needs? Rheumatology (Oxford) 59 (Suppl5) (2020) v52–v62.
[4] H.Y. Chun, Chung Jw Fau, H.-A. Kim, Kim Ha Fau, J.-M. Yun, Yun Jm Fau, J.-Y. Jeon, Jeon Jy Fau, Y.-M. Ye, Ye Ym Fau, S.-H. Kim, Kim Sh. Fau, H.-S. Park, Park Hs Fau, C.-H. Suh, C.H. Suh, Cytokine IL-6 and IL-10 as biomarkers in systemic lupus erythematosus, J. Clin. Immunol. 27 (5) (2007) 461–466.
[5] J. Liu, X. Wang, X. Yang, Q. Yan, S. Wang, W. Han, Investigating the role of angiogenesis in systemic lupus erythematosus, Lupus 24 (6) (2015) 621–627.
[6] Y. Liu, N. Tie, L. Bai, Serum levels of MDC and MMP-9 and the relationship between serum levels and disease activity in the patients with systemic lupus erythematosus, Pak. J. Med. Sci. 31 (4) (2015) 803–806.
[7] S.J. Deijns, J.C.A. Broen, N.D. Kruyt, C.D. Schubart, L. Andreoli, A. Tincani, M. Limper, The immunologic etiology of psychiatric manifestations in systemic lupus erythematosus: a narrative review on the role of the blood brain barrier, antibodies, cytokines and chemokines, Autoimmun. Rev. 19 (8) (2020), 102592.
[8] K. Ichinose, K. Arima, T. Ushigusa, A. Nishino, Y. Nakashima, T. Suzuki, Y. Horai, H. Nakajima, S.Y. Kawashiri, N. Iwamoto, et al., Distinguishing the cerebrospinal fluid cytokine profile in neuropsychiatric systemic lupus erythematosus from other autoimmune neurological diseases, Clin. Immunol. (Orlando, Fla) 157 (2) (2015) 114–120.
[9] T. Yoshio, H. Okamoto, K. Kurasawa, Y. Dei, S. Hirohata, S. Minota, IL-6, IL-8, IP-10, MCP-1 and G-CSF are significantly increased in cerebrospinal fluid but not in sera of patients with central neuropsychiatric lupus erythematosus, Lupus 25 (9) (2016) 997–1003.
[10] H. Jeltsch-David, S. Muller, Neuropsychiatric systemic lupus erythematosus: pathogenesis and biomarkers, Nat. Rev. Neurol. 10 (10) (2014) 579–596.
[11] S. Matus, P.V. Burgos, M. Bravo-Zehnder, R. Kraft, O.H. Porras, P. Farías, L. F. Barros, F. Torrealba, L. Massardo, S. Jacobelli, et al., Antiribosomal-P autoantibodies from psychiatric lupus target a novel neuronal surface protein causing calcium influx and apoptosis, J. Exp. Med. 204 (13) (2007) 3221–3234.
[12] M.Y. Choi, R.D. FitzPatrick, K. Buhler, M. Mahler, M.J. Fritzler, A review and meta-analysis of anti-ribosomal P autoantibodies in systemic lupus erythematosus, Autoimmun. Rev. 19 (3) (2020), 102463.
[13] J.A. Varley, M. Andersson, E. Grant, A. Berretta, M.A.-O. Zandi, V.A.-O. Bondet, D. A.-O. Duffy, D. Hunt, F.A.-O. Piehl, P.A.-O. Waters, et al., Absence of Neuronal Autoantibodies in Neuropsychiatric Systemic Lupus Erythematosus, Ann. Neurol. 88 (6) (2020) 1244–1250.
[14] G.T. Park, S.E. Lim, S.-I. Jang, M.I. Morasso, Suprabasin, a novel epidermal differentiation marker and potential cornified envelope precursor, J. Biol. Chem. 277 (47) (2002) 45195–45202.
[15] M.T. Alam, H. Fau Nagao-Kitamoto, N. Ohga, Ohga N. Fau, K. Akiyama, Akiyama K. Fau, N. Maishi, Maishi N. Fau, T. Kawamoto, Kawamoto T. Fau, N. Shinohara, Shinohara N. Fau, A. Taketomi, Taketomi A. Fau, M. Shindoh, Shindoh M. Fau, Y. Hida, Hida Y. Fau, K. Hida, et al., Suprabasin as a novel tumor endothelial cell marker, Cancer Sci. 105 (12) (2014) 1533–1540.
[16] K. Ichinose, K. Ohyama, K. Furukawa, O. Higuchi, A. Mukaino, K. Satoh, S. Nakane, T. Shimizu, M. Umeda, S. Fukui, et al., Novel anti-suprabasin antibodies may contribute to the pathogenesis of neuropsychiatric systemic lupus erythematosus, Clin. Immunol. (Orlando, Fla) 193 (2018) 123–130.
[17] E.M. Tan, A.S. Cohen, J.F. Fries, A.T. Masi, D.J. McShane, N.F. Rothfield, J. G. Schaller, N. Talal, R.J. Winchester, The 1982 revised criteria for the classification of systemic lupus erythematosus, Arthritis Rheum. 25 (11) (1982) 1271–1277.
[18] The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes, Arthritis Rheum. 42 (4) (1999) 599–608.

[19] S. Miyakis, M.D. Lockshin, T. Atsumi, D.W. Branch, R.L. Brey, R. Cervera, R. H. Derksen, PG DEG, T. Koike, P.L. Meroni, et al., International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS), J. Thromb. Haemost. JTH 4 (2) (2006) 295–306.
[20] C. Bombardier, D.D. Gladman, M.B. Urowitz, D. Caron, C.H. Chang, Derivation of the SLEDAI. A disease activity index for lupus patients. The committee on prognosis studies in SLE, Arthritis Rheum. 35 (6) (1992) 630–640.
[21] J.J. Weening, V.D. D'Agati, M.M. Schwartz, S.V. Seshan, C.E. Alpers, G.B. Appel, J. E. Balow, J.A.N.A. Bruijn, T. Cook, F. Ferrario, et al., The classification of glomerulonephritis in systemic lupus erythematosus revisited, Kidney Int. 65 (2) (2004) 521–530.
[22] K.L. Jones, L.A. Croen, C.K. Yoshida, L. Heuer, R. Hansen, O. Zerbo, G. N. DeLorenze, M. Kharrazi, R. Yolken, P. Ashwood, et al., Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation, Mol. Psychiatry 22 (2) (2017) 273–279.
[23] V.J. Yohai, High breakdown-point and high efficiency robust estimates for regression, Ann. Stat. 15 (1987) 642–656.
[24] E. Vradi, T. Jaki, R. Vonk, W. Brannath, A Bayesian model to estimate the cutoff and the clinical utility of a biomarker assay, Stat. Methods Med. Res. 28 (8) (2019) 2538–2556.
[25] C. Ye, Y. Yang, Y. Yang, Sparsity oriented importance learning for high-dimensional linear regression, J. Am. Stat. Assoc. 113 (524) (2018) 1797–1812.
[26] C. Ye, Y. Yang, Y. Yang, SOIL: Sparsity Oriented Importance Learning. R package version 1.1. https://CRAN.R-project.org/package=SOIL, 2017.
[27] Team RC, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2019. https://www.R-project.org/.
[28] T. Matsui, F. Hayashi-Kisumi, Y. Kinoshita, S. Katahira, K. Morita, Y. Miyachi, Y. Ono, T. Imai, Y. Tanigawa, T. Komiya, et al., Identification of novel keratinocyte-secreted peptides dermokine-α/−β and a new stratified epithelium-secreted protein gene complex on human chromosome 19q13.1, Genomics 84 (2) (2004) 384–397.
[29] S. Hubackova, M. Pribyl, L. Kyjacova, A. Moudra, R. Dzijak, B. Salovska, H. Strnad, V. Tambor, T. Imrichova, J. Svec, et al., Interferon-regulated suprabasin is essential for stress-induced stem-like cell conversion and therapy resistance of human malignancies, Mol. Oncol. 13 (7) (2019) 1467–1489.
[30] J.K. Kruschke, Rejecting or accepting parameter values in Bayesian estimation, Adv. Methods Pract. Psychol. Sci. 1 (2) (2018) 270–280.
[31] C. Hammer, B. Stepniak, A. Schneider, S. Papiol, M. Tantra, M. Begemann, A. L. Sirén, L.A. Pardo, S. Sperling, S. Mohd Jofrry, et al., Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity, Mol. Psychiatry 19 (10) (2014) 1143–1149.
[32] N. Schwartz, A.D. Stock, C.A.-O. Putterman, Neuropsychiatric lupus: new mechanistic insights and future treatment directions, Nat. Rev. Rheumatol. 15 (3) (2019 Mar) 137–152.
[33] J.F. Carvalho, Blank M. Fau, Y. Shoenfeld, Y. Shoenfeld, Vascular endothelial growth factor (VEGF) in autoimmune diseases, J. Clin. Immunol. 27 (3) (2007) 246–256.
[34] N. Ferrara, Vascular endothelial growth factor: basic science and clinical Progress, Endocr. Rev. 25 (4) (2004) 581–611.
[35] M.J. Cross, L. Claesson-Welsh, FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition, Trends Pharmacol. Sci. 22 (4) (2001) 201–207.
[36] S. Hirohata, H. Kikuchi, Role of serum IL-6 in neuropsychiatric systemic lupus erythematosus, ACR Open Rheumatol. 3 (1) (2021) 42–49.
[37] C.K. Smith, M.J. Kaplan, The role of neutrophils in the pathogenesis of systemic lupus erythematosus, Curr. Opin. Rheumatol. 27 (5) (2015).
[38] J.-B. Wang, H. Li, L.-L. Wang, H.-D. Liang, L. Zhao, J. Dong, Role of IL-1β, IL-6, IL-8 and IFN-γ in pathogenesis of central nervous system neuropsychiatric systemic lupus erythematous, Int. J. Clin. Exp. Med. 8 (9) (2015) 16658–16663.
[39] M. Figueiredo-Braga, Cornaby C. Fau, A. Cortez, Cortez A. Fau, M. Bernardes, Bernardes M. Fau, G. Terroso, Terroso G. Fau, M. Figueiredo, Figueiredo M. Fau, Mesquita CDS, Mesquita Cds Fau, L. Costa, Costa L. Fau, B.D. Poole, Depression and anxiety in systemic lupus erythematosus: the crosstalk between immunological, clinical, and psychosocial factors, Medicine (Baltimore) 97 (28) (2018), e11376.
[40] N. Berkman, M. Fau John, G. Roesems, Roesems G. Fau, P.J. Jose, Jose Pj Fau, P. J. Barnes, Barnes Pj Fau, K.F. Chung, K.F. Chung, Inhibition of macrophage inflammatory protein-1 alpha expression by IL-10. Differential sensitivities in human blood monocytes and alveolar macrophages, J. Immunol. 155 (9) (1995) 4412–4418.
[41] J. Park, W. Jang, H.S. Park, K.H. Park, S.-K. Kwok, S.-H. Park, E.-J. Oh, Cytokine clusters as potential diagnostic markers of disease activity and renal involvement in systemic lupus erythematosus, J. Int. Med. Res. 48 (6) (2020), 300060520926882–300060520926882.
[42] R.K. Vakkalanka, Woo C. Fau, K.A. Kirou, Kirou Ka Fau, M. Koshy, Koshy M. Fau, D. Berger, Berger D. Fau, M.K. Crow, M.K. Crow, Elevated levels and functional capacity of soluble CD40 ligand in systemic lupus erythematosus sera, Arthritis Rheum. 42 (5) (1999 May) 871–881.
[43] A. Goules, Tzioufas Ag Fau, M.N. Manousakis, Manousakis Mn Fau, K.A. Kirou, Kirou Ka Fau, M.K. Crow, Crow Mk Fau, J.G. Routsias, J.G. Routsias, Elevated levels of soluble CD40 ligand (sCD40L) in serum of patients with systemic autoimmune diseases, J. Autoimmun. 26 (3) (2006) 165–171.
[44] M. Postal, A.T. Lapa, N.A. Sinicato, Peliçari K. de Oliveira, F.A. Peres, L.T. L. Costallat, P.T. Fernandes, R. Marini, S. Appenzeller, Depressive symptoms are associated with tumor necrosis factor alpha in systemic lupus erythematosus, J. Neuroinflammation 13 (1) (2016) 5.
[45] R.-X. Leng, H.-F. Pan, D.-Q. Ye, Y. Xu, Potential roles of IL-9 in the pathogenesis of systemic lupus erythematosus, Am. J. Clin. Exp. Immunol. 1 (1) (2012) 28–32.
[46] M. Di Nicola, A. Cattaneo, N. Hepgul, M. Di Forti, K.J. Aitchison, L. Janiri, R. M. Murray, P. Dazzan, C.M. Pariante, V. Mondelli, Serum and gene expression profile of cytokines in first-episode psychosis, Brain Behav. Immun. 31 (2013) 90–95.