A novel nomogram based on the identification of sTREM2 as a biomarker to predict developing neuropsychiatric systemic lupus erythematosus in lupus patients

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Abstract

Objective

This study aimed to identify potential biomarkers and construct a nomogram able to predict the development of neuropsychiatric systemic lupus erythematosus (NPSLE) among SLE patients.

Methods

Using bioinformatics analysis, TREM2 was identified as an upregulated gene in NPSLE, participating in various pathological pathways of NPSLE. This study included 80 NPSLE patients and three matched SLE controls with no neuropsychiatric events (non-NPSLE controls) for each of the NPSLE patients. Both serum and cerebrospinal fluid (CSF) concentrations of soluble TREM2 (sTREM2) were assessed. The diagnostic capability of sTREM2 for NPSLE was evaluated using the receiver-operating characteristic curve (ROC curves). The study subsequently integrated a substantial volume of clinical data. Following missing data imputation, patients were randomly allocated to either the training set or the validation set. The Boruta algorithm and Multiple analyses were utilised for constructing the nomogram. Diagnostic performance was assessed using ROC curves, the Hosmer-Lemeshow test, and clinical decision curves.

Results

sTREM2 levels were notably elevated in both serum and CSF of NPSLE patients compared to non-NPSLE controls. Serum TREM2 concentrations correlated with NPSLE severity and neuropsychiatric state. Notably, higher SLE Disease Activity Index (SLEDAI), increased systemic lupus international collaborating clinics (SLICC)/ACR damage index (SDI), prolonged activated partial thromboplastin time (APTT), a higher serum B cells, and elevated serum sTREM2 levels emerged as significant predictors for NPSLE.

Conclusion

sTREM2 presents as a promising biomarker for NPSLE diagnosis. The nomogram that includes serum sTREM2 level as one of the predictors is effective for distinguishing NPSLE from non-NPSLE patients.

Key words

neuropsychiatric systemic lupus erythematosus, sTREM2, biomarker, nomogram

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Received on December 2, 2024; accepted in revised form on March 17, 2025.

Funding: this work was supported by grants from the Program of Innovative and Entrepreneurial Talent of Jiangsu Province ([2020]30099) and the Young Scholars Fostering Fund of the First Affiliated Hospital with Nanjing Medical University (PY2021033).

Competing interests: none declared.

Introduction

Neuropsychiatric systemic lupus erythematosus (NPSLE) is a subtype of SLE that primarily affects the nervous system, presenting with a range of symptoms, such as headache, cognitive impairment, seizures, and more (1, 2). The reported prevalence of NPSLE is highly variable, with rates ranging from 28% to 60% in different cohorts (3-6), and can lead to disability or even death (7, 8). Diagnosis of NPSLE is challenging since it is difficult to distinguish NPSLE from other neuropsychiatric conditions with different aetiologies (9). The mechanisms underlying NPSLE remain unclear and have been considered to involve varieties of autoantibodies, cytokines, and immune complexes (10, 11). The absence of a specific biomarker for NPSLE is one of the important reasons that make the diagnosis difficult (12).

At present, given the complex nature of NPSLE and the need for better diagnostic tools, we employed bioinformatics analysis to screen potential biomarkers that may be beneficial for diagnosing NPSLE. We found that *TREM2* is upregulated in NPSLE and participates in NPSLE pathological pathways, including microglia activation, microglia migration, and synaptic pruning. Triggering receptor expressed on myeloid cells 2 (TREM2), a surface receptor of microglia, plays important roles in microglial functions including modulation of neuroinflammation and phagocytosis (13, 14). TREM2 is released into the extracellular space as a soluble form (sTREM2) and can be detected in the cerebrospinal fluid (CSF) and peripheral blood (15, 16). Previous study proved that CSF sTREM2 serves as a surrogate measure of microglial activity (17) and that microglia activation exerts important roles in the pathogenesis of NPSLE (18). Therefore, this study aimed to evaluate, for the first time, the potential of sTREM2 as a biomarker for identifying SLE patients with neuropsychiatric manifestations. However, it is difficult to use a single test for diagnosing NPSLE with high sensitivity and specificity. Therefore, it is necessary to develop a model to screen NPSLE.

In this study, we investigated the predictors for NPSLE and aimed to find a specific combination of sTREM2, clinical, and laboratory parameters in NPSLE that can be distinguished from the profiles of SLE. The diagnostic nomogram model we constructed will help clinicians identify patients at high risk of developing NPSLE.

Patients and methods

Study design

We screened the upregulated genes in NPSLE using mRNA expression profile data from the Gene Expression Omnibus (GEO) and identified TREM2 as the candidate. We enrolled 80 NPSLE patients and selected three age-matched and gender-matched SLE patients with no neuropsychiatric (NP) events for each NPSLE patient (non-NPSLE controls). We measured the soluble TREM2 levels in the serum and CSF of these patients and collected their clinical data. Patients were randomly assigned into training and validation sets at 7:3. The Boruta algorithm and multivariate analysis were used to select important variables for developing the nomogram model. The ROC curve, calibration curves, Hosmer-Lemeshow test and decision curve analysis (DCA) were performed to validate the model.

RNA-seq data analysis

A data set containing mRNA expression profiles of 12 NPSLE samples from MRL/lpr mice and 6 control samples from MRL/MpJ-Fas+/+ (MRL/+) mice was downloaded from the (GEO; accession number: GSE99030; www.ncbi.nlm.nih.gov/geo/). The choroid plexus from these mice was isolated, flash-frozen in liquid nitrogen, and subsequently used for RNA isolation and further analysis. GSE99030 is based on the GPL17021 (Illumina HiSeq 2500) platform. To determine differentially expressed genes (DEGs), a filtering threshold of p-value of <0.05 and $|\log FC| > 1.0$ was applied. The heatmap visualisation of DEGs was generated by using Morpheus software (https://software.broadinstitute.org/morpheus/) and clustered by k-means clustering based on their expression patterns. We set K at 2, and clusters are calculated using Pearson correlation.

Gene ontology term enrichment analysis

The biological significance of DEGs was explored by using gene ontology (GO) term enrichment analysis of biological processes (http://geneontology.org/page/go-enrichment-analysis). The enrichment bubble plots and the Venn diagram were created by using an online platform SRplot. And the SRplot web server is now freely available at http://www.bioinformatics.com.cn/SRplot.

Patients and samples

Patients diagnosed with SLE hospitalised in the Department of Rheumatology of the First Affiliated Hospital with Nanjing Medical University, Nanjing, China between September 2019 and May 2024, according to the American College of Rheumatology (ACR) 2012 revised criteria formed the population of this study. The enrolment window extended from 6 months prior to the diagnosis of SLE up to the actual enrolment date. Neuropsychiatric (NP) events were characterised within this window using the 1999 ACR definitions for 19 NPSLE syndromes (2), and the diagnosis of NPSLE was carefully made by rheumatologists and neurologists. In total, 80 NPSLE patients were enrolled in the present study. In addition, we selected three age-matched and sex-matched controls who had no record of NP events and fulfilling the American College of Rheumatology (ACR) 2012 revised criteria for the classification of SLE (non-NPSLE controls) (19) for each of 80 NPSLE patients. Additionally, 7 healthy controls and 6 patients with NP symptoms from connective tissue diseases were also included (CTD-NP controls). Among the CTD-NP controls, there were 2 cases of Sjögren's syndrome with neuromyelitis optica spectrum disorder (NMOSD), 1 case of Behçet's disease with depression, 1 case of dermatomyositis with autoimmune encephalitis, 1 case of rheumatoid arthritis with Guillain-Barré syndrome, and 1 case of giant cell arteritis.

According to the reversible multistate Makovian model for NP status for NPSLE patients (20), 68 NPSLE patients were with new/ongoing NP events and 27 NPSLE patients were with resolved NP events. NPSLE patients with new/ongoing NP events included peripheral nervous system (PNS) manifestations (n=7) and central nervous system (CNS) manifestations (n=61). The patients with CNS manifestations included focal (n=35) and diffuse (n=26) NPSLE. The patients with diffuse NPSLE included those with ACS (n=10) and dNPSLE excluding ACS (non-ACS) (n=16), including anxiety disorder, cognitive dysfunction, mood disorder, and psychosis. The details are shown in Supplementary Table S1.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committees of the First Affiliated Hospital with Nanjing Medical University (Ethical approval no.: 2021-SR-464). Informed consent was obtained from all the study participants. CSF samples were obtained from the participants by lumbar puncture; CSF of 9 non-NPSLE controls and 36 NPSLE patients were obtained. The serum samples of all patients were collected on the day of admission or diagnosis.

Data collection

A standard case report form was established to retrospectively collect the demographic data; clinical characteristics data: duration of SLE, laboratory data and immunosuppressive treatment options.

Measurement of serum and CSF concentrations of sTREM2

The level of sTREM2 was determined using an enzyme linked immunosorbent assay (ELISA). After the freeze-thaw cycles, the sTREM2 levels in CSF and plasma were measured using the Human TREM2 DuoSet ELISA (R&D Systems: no. DY1828-05). Briefly, anti-human TREM2 antibodies (no. 844598, R&D) were used as capture antibodies, and the samples were incubated overnight at room temperature. For detection, a biotinylated mouse antihuman TREM2 (no. 844599, R&D) was incubated for 2h at room temperature after the addition of the samples and recombinant human TREM2 standard (no. 844600, R&D). Horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Caltag, San Francisco, CA) and the substrate 2,2-azinobis-3-ethylbenzothiazoline sulfonic acid was used for detection and visualisation. The optical density at 450 nm was used for data analysis.

Statistical analyses

Descriptive statistics contained continuous and categorical variables. The normal distribution of continuous variables was presented by mean±SD. The non-normal distribution was described by median (IQR). Comparison of categorical variables was performed by the Chi-square test, and comparison of continuous variables was performed by t-test (for normally distributed data) or Mann-Whitney U-tests (for non-normally distributed data). As a primary analysis, we tested differences in mean sTREM2 levels between NPSLE patients and non-NPSLE patients using Student's *t* test. For secondary analyses, the area under the curve (AUC) derived from ROC analysis was calculated for sTREM2 levels.

To develop and validate the diagnostic model for NPSLE, 80 NPSLE patients and 240 matched non-NPSLE controls were randomly assigned into training and validation sets at a ratio of 7:3. To fill in the missing values of clinical characteristics, we utilised multiple imputation ways. The Boruta algorithm was used to select important variables. Boruta, a feature selection method based on random forests, evaluates the relevance and independent contribution of each feature by generating shadow features, which are randomly permuted versions of the original features. The algorithm compares the importance scores of the original features with those of the shadow features. This approach minimises the error of the random forest model and ultimately yields a subset of optimal features. Forward stepwise regression was applied in the procedure to reduce multicollinearity. Finally, Variables with $p<0.05$ were included in the multivariate analysis to develop the nomogram model. The discriminatory performance of the model was assessed using ROC curves and AUC statistics. Model calibration was evaluated with calibration curves and the Hosmer-Lemeshow test, and clinical utility was assessed us-

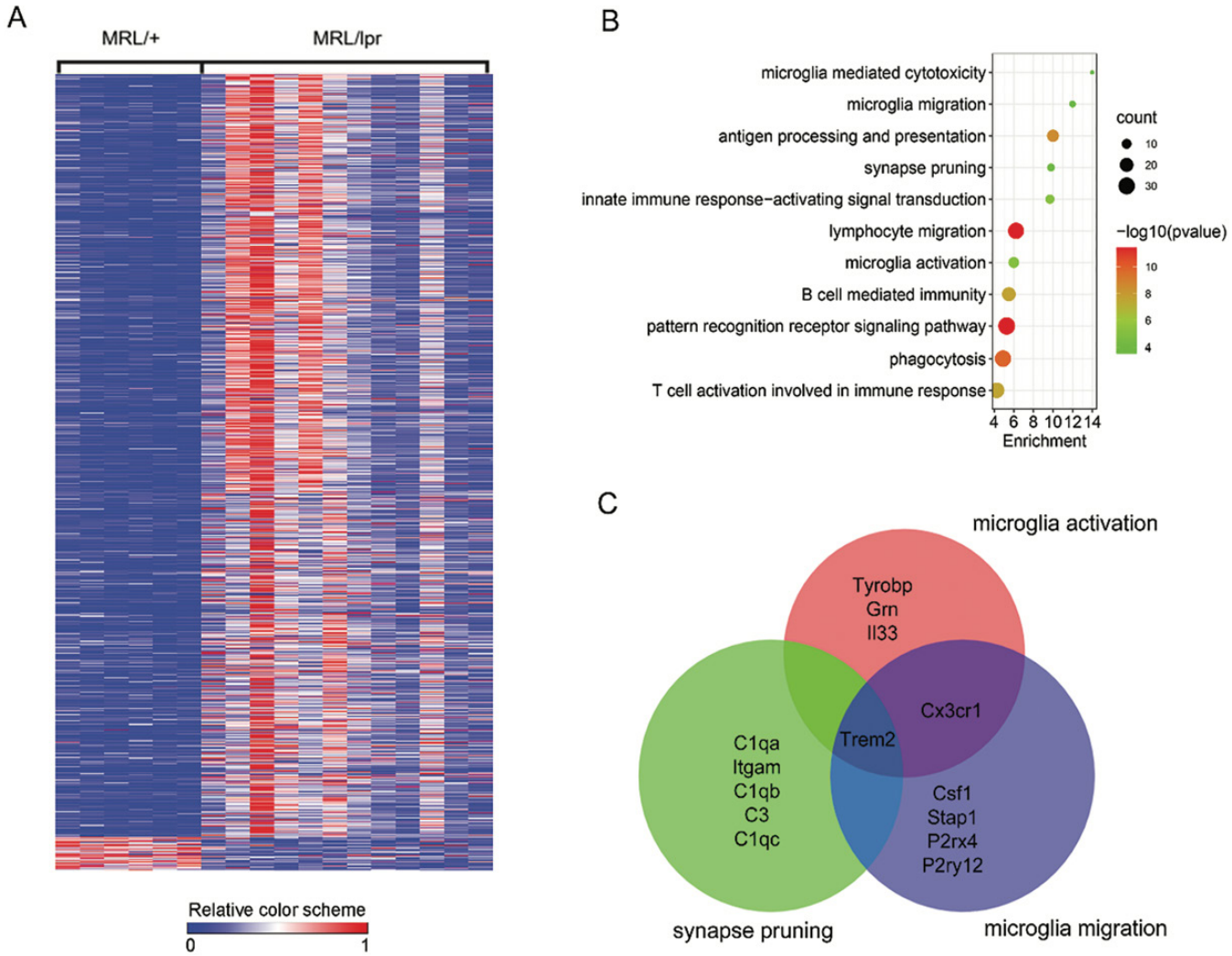

Fig. 1. Analysis of DEGs of GSE99030 identified TREM2 as a potential biomarker for NPSLE.
A: Heat maps of 2122 DEGs in the choroid plexus of MRL/lpr *vs*. MRL/+ mice.
B: GO enrichment analysis of DEGs.
C: Venn diagram shows TREM2 is the intersection gene in three NPSLE pathological pathways (microglia activation, microglia migration and synaptic pruning).
DEG: differentially expressed genes; NPSLE: neuropsychiatric systemic lupus erythematosus; TREM2: triggering receptor expressed on myeloid cells 2.

ing DCA. The flowchart is provided in Supplementary Figure S1. The significance level was set at $p<0.05$. Statistical analysis was performed using SPSS v. 27.0 (SPSS Inc., Chicago, IL, USA), GraphPad Prism software 9 (San Diego, CA, USA) and R 4.4.1 along with Mice, Boruta, rms, caret, pROC, ResourceSelection and ggDCA packages. (R Foundation for Statistical Computing, 128 Vienna, Austria).

Results

Elevated TREM2 expression in the brain of MRL/lpr mice is linked to the regulation of NPSLE pathological pathways

The NPSLE mRNA expression profiling GSE99030 was analysed and the results are shown in Figure 1. Overall, 2122 DEGs were identified, including 2033 up-regulated genes and 89 down-regulated genes (Fig. 1A). GO enrichment analysis of these DEGs revealed that the up-regulated genes were primarily associated with microglia regulation, synaptic pruning, and lymphocyte activity (Fig. 1B). Notably, TREM2 expression was found to be elevated in the brains of MRL/lpr mice (NPSLE models) compared to MRL/+ mice. TREM2 emerged as a key gene intersecting various NPSLE pathological pathways, including microglia activation, migration, and synaptic pruning (Fig. 1C). Hence, our findings suggest that TREM2 is upregulated in NPSLE and could also serve as a potential biomarker for NPSLE.

Baseline characteristics of the study population

In this study, 2217 SLE patients were included, comprising 80 NPSLE patients and 2137 SLE patients without NP events. We analysed the demographic and clinical data of 80 NPSLE patients and 240 matched non-NPSLE patients. Table I shows the baseline characteristics of the NPSLE and non-NPSLE controls. Significant differences were found in SLEDAI, SDI, serum sTREM2, red blood cell (RBC), haemoglobin, lymphocytes, eosinophil, T helper cell, T lymphocyte, B cells, aspartate transaminase (AST), lactate dehydrogenase (LDH), activated partial thromboplastin time (APTT), globulin (Glb), albumin/globulin ratio (A/G ra-

Table I. Baseline variables of the NPSLE patients and matched non-NPSLE controls.

Characteristic	Total (n=320)	NPSLE (n=80)	non-NPSLE (n=240)	*p*-value
Age, year	36.5 (28,52)	39.79 (27.51)	39.19 (28,52)	0.811
Gender (male:female)	28:292	7:73	21:219	1.000
Duration, month	36 (2,108)	60.12 (2,105)	69.74 (2,120)	0.288
SLEDAI	**12 (6,17.75)**	**20 (14,26)**	**9 (6,15)**	**<0.001***
SDI	**1 (0,1)**	**1.51 (0,2)**	**0.63 (0,1)**	**<0.001***
Serum sTREM2, pg/ml	**803.51 (479.84,1237.1)**	**1231.93 (847.8,1426.9)**	**821.96 (430.3,1033)**	**<0.001***
WBC, $\times10^9$/L	4.88 (3.25,6.79)	5.7761 (3.4,7.0)	5.484 (3.2,6.8)	0.482
RBC, $\times10^9$/L	**3.7 (3.12,4.16)**	**3.48 (2.74,4.13)**	**3.7 (3.25,4.08)**	**0.007***
Haemoglobin, g/L	**104.56 ± 22.04**	**98.54 ± 24.25**	**106.51 ± 20.91**	**0.005***
Platelet, $\times10^9$/L	180 (102.25,232)	161 (94,235)	181 (91,235)	0.26
Monocytes, $\times10^9$/L	0.35 (0.2,0.52)	0.36 (0.17,0.51)	0.36 (0.18,0.52)	0.458
Reticulocyte, %	1.84 (1.3,2.5)	2.16 (1.29,3.36)	1.74 (1.31,2.35)	0.064
Neutrophil, $\times10^9$/L	3.18 (2.06,5.02)	3.66 (2.39,5.28)	2.57 (1.83,4.57)	0.117
Eosinophil, **$\times10^9$/L**	**0.01 (0,0.04)**	**0 (0,0.01)**	**0.01 (0,0.04)**	**0.001***
Basophils, $\times10^9$/L	0.01 (0,0.02)	0.01 (0,0.02)	0.01 (0,0.02)	0.099
Lymphocyte, %	**19.4 (11.4,28.1)**	**15.4 (7.3,26.1)**	**21.3 (11.4,28.9)**	**0.002***
T helper cell, %	**35 (26.1,42.8)**	**32.2 (25.1,40.1)**	**34.1 (25.7,43.7)**	**0.049***
Cytotoxic T cell, %	33.4 (26.4,43.3)	36.37 (23.7,45.1)	32.9 (25.6,44.3)	0.546
T lymphocyte, %	**75.55 (65.5,84.18)**	**71 (60.9,82)**	**76.6 (66.6,83.9)**	**0.02***
Nature killer cells, %	4.7 (2.6,8.9)	4 (1.8,8.9)	4.8 (2.8,8.9)	0.114
B cells, %	**14.1 (7.3,24.75)**	**19.3 (11.4,30)**	**13.3 (6.7,23.6)**	**0.01***
ALT, U/L	18.35 (12.4,35.7)	23.5 (13.1,46.4)	17.4 (12.2,30.4)	0.064
AST, U/L	**23.2 (17.2,39.15)**	**28.3 (18.6,52.5)**	**22.3 (16.4,32.6)**	**0.007***
LDH, U/L	**226 (178,305.75)**	**279 (199,387)**	**219 (169,280)**	**<0.001***
TP,g/L	62.84 ± 11.19	64.36 ± 11.24	62.33 ± 11.14	0.16
Glb, g/L	**29.85 (25.9,34.68)**	**32.5 (26.1,36.1)**	**29.2 (25.9,34.5)**	**0.011***
ALB, g/L	32.25 (27.93,36.3)	31.35 ± 6.3	23.07 ± 5.88	0.49
A/G ratio, %	**1.1 (0.9,1.3)**	**0.9 (0.8,1.3)**	**1.1 (0.9,1.3)**	**0.025***
Scr, μmol/L	56.35 (45.88,69.18)	59.5 (48.2,80.1)	54.5 (45.2,69.1)	0.164
BUN,mmol/L	**5.53 (4.17,8.01)**	**6.52 (4.65,10.28)**	**5.54 (4.38,7.57)**	**0.003***
PT, sec	11.5 (10.9,12.1)	11.5 (11.1,12.4)	11.4 (10.8,11.9)	0.089
APTT, sec	**27.2 (24.9,30.6)**	**27.8 (25.1,33.5)**	**26.6 (24.8,29.5)**	**0.01***
TT, sec	17.3 (16.6,18.1)	17.3 (16.3,19.1)	17.3 (16.5,18)	0.241
FIB, g/L	**2.71 (2.08,3.54)**	**2.62 (1.92,3.14)**	**2.77 (2.14,3.6)**	**0.045***
Anti-dsDNA antibody	160 (50.00%)	36 (45.00%)	124 (51.67%)	0.406
ACL positive	115 (35.94%)	31 (38.75%)	84 (35.00%)	0.591
IgG, g/L	**14.6 (11.2,18.6)**	**15.8 (12.4,21.1)**	**14.3 (10.8,18.4)**	**0.008***
IgA, g/L	2.59 (1.75,3.57)	2.4 (1.55,3.41)	2.56 (1.99,3.62)	0.515
IgM, g/L	0.9 (0.61,1.49)	0.94 (0.69,1.96)	0.89 (0.57,1.51)	0.095
C3, g/L	0.52 (0.37,0.68)	0.46 (0.28,0.65)	0.55 (0.41,0.67)	0.063
C4, g/L	**0.1 (0.05,0.16)**	**0.09 (0.03,0.14)**	**0.11 (0.06,0.17)**	**0.002***
CRP, mg/L	4.73 (1.82,13.3)	5.62 (2.05,28.7)	3.94 (1.76,12.1)	0.153
ESR, mm/h	30 (13,51)	29 (13,59)	28 (13,50)	0.889
Immunosuppressive treatment				
Glucocorticoid				0.696
yes	190 (59.38%)	46 (57.5%)	144 (60%)	
no	130 (40.63%)	34 (42.5%)	96 (40%)	
Anti-malarials				0.245
yes	160 (50.00%)	35 (43.8%)	125 (52.1%)	
no	160 (50.00%)	45 (56.2%)	115 (47.9)	
Immunosuppressant				0.154
yes	143 (44.69%)	30 (37.5%)	113 (47.1%)	
no	177 (55.31%)	80 (62.5%)	127 (52.9%)	

p-value for between-group comparisons. Bold text highlights significant values. *Values statistically significant at $p<0.05$.
ACL: anticardiolipin antibody; ALB: albumin; ALT: alanine transaminase; APTT: activated partial thromboplastin time; AST: aspartate transaminase; A/G ratio: albumin/globulin ratio; BUN: blood urea nitrogen; CK: creatine kinase; CSF: cerebrospinal fluid; CRP: C-reactive protein; C3: complement 3; C4: complement 4; dsDNA: double-stranded DNA; ESR: erythrocyte sedimentation rate; FIB: fibrinogen; Glb: globulin; HB: haemoglobin; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; IQR (interquartile ranges); LDH: lactate dehydrogenase; NPSLE: neuropsychiatric systemic lupus erythematosus; Non-NPSLE: SLE with no neuropsychiatric events; PT: prothrombin time; RBC: red blood cell; Scr: serum creatinine; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SDI: Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology Damage Index; sTREM2: soluble triggering receptor expressed on myeloid cells 2; TP: total protein; TT: thrombin time; WBC: white blood cell.

tio), Blood Urea Nitrogen (BUN), fibrinogen (FIB), immunoglobulin (IgG), and complement 4 (C4) between the two groups ($p<0.05$).

Elevated levels of soluble TREM2 (sTREM2) in serum and CSF of NPSLE patients

Compared to healthy controls (HC, n=7) and matched control of SLE patients without NP events (non-NPSLE, n=240), sTREM2 levels were significantly elevated in serum of NPSLE

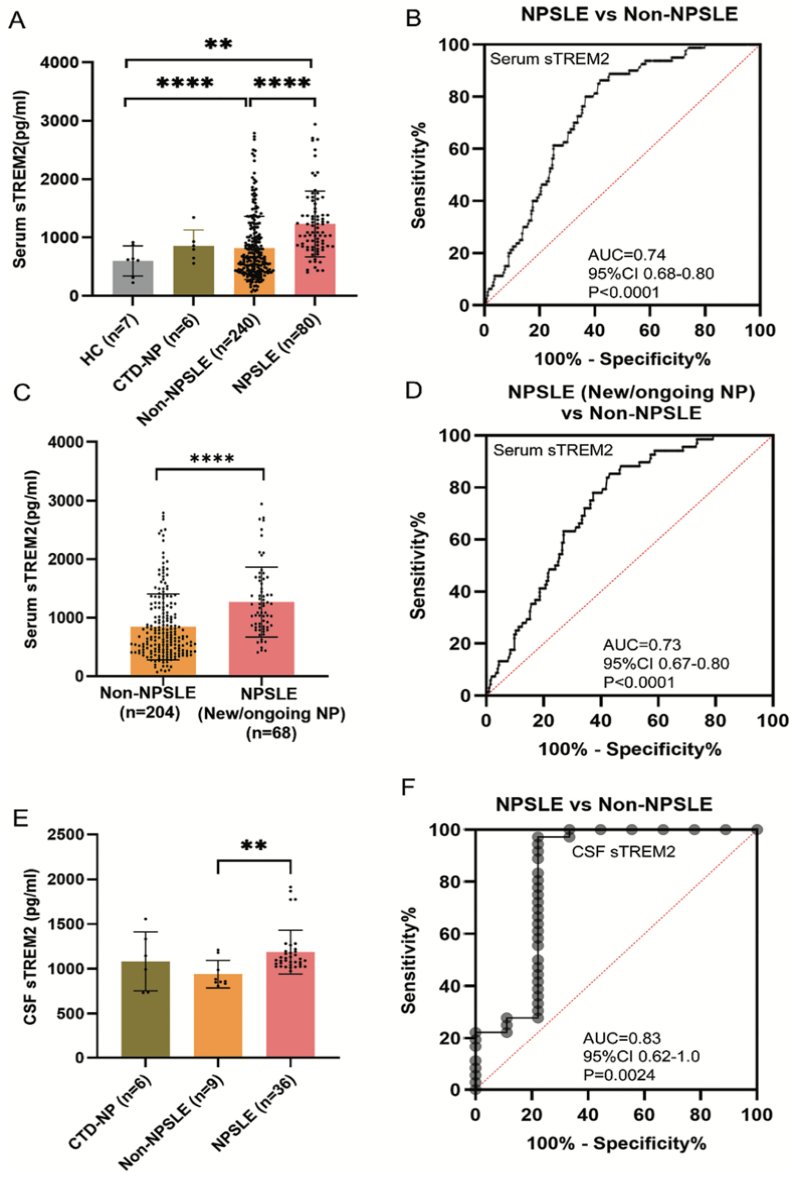

Fig. 2. CSF and serum concentrations of sTREM2 in the control individuals and NPSLE patients.
A: Serum concentration of sTREM2 in healthy controls (n=7), CTD-NP controls (n=6), non-NPSLE controls (n=240) and NPSLE patients (n=80)).
B: ROC curve analysis of serum sTREM2 in NPSLE and matched non-NPSLE controls.
C: Serum concentration of sTREM2 in NPSLE with new/ongoing NP events (n=68) and matched non-NPSLE controls (n=204).
D: ROC curve analysis of serum sTREM2 in NPSLE with new/ongoing NP events and matched non-NPSLE controls.
E: CSF concentration of sTREM2 in CTD-NP controls (n=6), non-NPSLE controls (n=9) and NPSLE patients (n=36).
Statistical analysis was performed using the Mann-Whitney U-test. $^{**}p<0.01$. $^{****}p<0.0001$.
CSF: cerebrospinal fluid; NP: neuropsychiatric; NPSLE: neuropsychiatric systemic lupus erythematosus; Non-NPSLE: SLE with no neuropsychiatric events; ROC: receiver operating characteristic; sTREM2: soluble triggering receptor expressed on myeloid cells 2.

patients (n=80). However, there were no significant differences in serum sTREM2 levels between healthy controls and CTD-NP controls (n=6) or between CTD-NP controls and NPSLE patients (Fig. 2A). ROC curve analysis was employed to evaluate the diagnostic potential of serum sTREM2. The results indicated that serum sTREM2 possessed a greater diagnostic utility for the NPSLE group, achieving an AUC of 0.74 (95%CI: 0.68–0.80) with a sensitivity of 86.25% and a specificity of 57.92%. The diagnostic threshold was determined to be ≥755.0 pg/ml (Fig. 2B). We also further analysed SLE patients with new/ongoing NP events (NPSLE [new/ongoing NP]) in considering different NP states. Results indicated that sTREM2 levels in the serum of NPSLE (new/ongoing NP) were higher in compared to the matched control of non-NPSLE controls (Fig. 2C). The AUC for this subgroup was 0.73 (95%CI: 0.67–0.80) with a diagnostic cut-off of ≥755.0 pg/ml, having a sensitivity of 85.29% and specificity of 57.35% (Fig. 2D).

In addition, NPSLE patients (n=36) exhibited elevated sTREM2 levels in their CSF compared to the non-NPSLE controls (n=9). However, there were no significant differences in CSF sTREM2 levels between CTD-NP controls (n=6) and non-NPSLE or between CTD-NP controls and NPSLE patients (Figure 2E). The CSF sTREM2 levels showed good discriminatory capacity for NPSLE (AUC: 0.83, 95% CI: 0.62–1.0). A diagnostic threshold of ≥1001 pg/ml was found to have a sensitivity of 97.22% and a specificity of 77.78% in identifying NPSLE (Fig. 2F).

Correlation between serum sTREM2 concentrations and NPSLE severity

We evaluated the serum and CSF concentrations of sTREM2 across various NPSLE subgroups. Notably, serum sTREM2 levels did not significantly differ between SLE patients with peripheral nervous system (PNS) involvement and those with central nervous system (CNS) manifestations. No significant difference was observed between patients with focal NPSLE and those with diffuse NPSLE (Fig. 3A-B). However, serum sTREM2 concentrations were elevated in patients with acute confusional state (ACS) NPSLE compared to both focal NPSLE patients and non-ACS diffuse NPSLE patients (Fig. 3C). However, results showed that there was no significant difference in CSF concentration of sTREM2 across different subgroups of NPSLE patients (Fig. 3D-F).

Association between serum sTREM2 concentrations and neuropsychiatric (NP) states in NPSLE patients

We enrolled a total of 68 SLE patients experiencing new or ongoing NP events

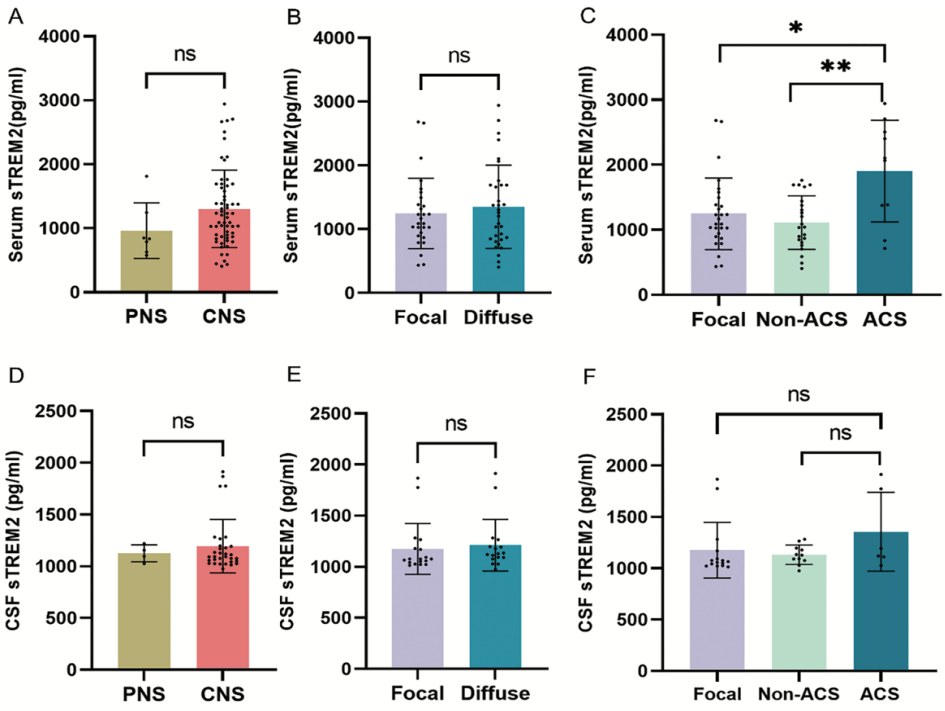

Fig. 3. Serum and CSF concentrations of sTREM2 in NPSLE patients with different NP events.
A: No significant difference in serum sTREM2 concentration was observed between NPSLE patients with PNS manifestations (n=7) and CNS manifestations (n=61).
B: No significant difference in serum sTREM2 concentration was observed between focal NPSLE (n=28) and diffuse NPSLE (n=33).
C: Serum sTREM2 concentrations were significantly higher in the ACS diffuse NPSLE (n=10) than in the focal NPSLE (n=28) or non-ACS diffuse NPLSE (n=23).
D: No significant difference in CSF sTREM2 concentration was observed between NPSLE patients with PNS manifestations (n=4) and CNS manifestations (n=32).
E): No significant difference in CSF sTREM2 concentration was observed between focal NPSLE (n=15) and diffuse NPSLE (n=17).
F: No significant difference in CSF sTREM2 concentration was observed among focal NPSLE (n=15), non-ACS diffuse NPLSE (n=11) and ACS diffuse NPSLE (n=6).
Statistical analysis was performed using the Mann-Whitney U-test. *p<0.05, **p<0.01.
ACS: acute confusional state; CSF: cerebrospinal fluid; CNS: central nervous system; non-ACS: diffuse NPSLE other than ACS; ns: not significant; PNS: peripheral nervous system; sTREM2: soluble triggering receptor expressed on myeloid cells 2.

and another 28 SLE patients who had resolved NP events. Among the former group, 16 eventually showed complete resolution of NP events post-treatment. Intriguingly, serum sTREM2 concentrations were found to be lower in SLE patients with resolved NP events when compared to those with new or ongoing NP events (Fig. 4A). Furthermore, a paired comparison indicated a notable decrease in serum sTREM2 levels upon resolution of the new/ongoing NP events post-treatment (Fig. 4B).

Construction and validation of a new model and nomogram for NPSLE

In order to construct the NPSLE diagnostic model, 320 patients were randomly divided into the training set (n=224) and the validation set (n=96). First, to fill in the missing values of clinical characteristics, we utilised multiple imputation ways. The Boruta algorithm was used to select important variables. Second, multivariate logistic regression analyses were performed on the Boruta algorithm screening results (Suppl. Fig. S2) to study associations between NPSLE patients and non-NPSLE controls, which included SLEDAI, SDI, serum sTREM2, APTT, RBC, B cells, NK cells, IgG. Variables with p<0.05 were included in the multivariate analysis to develop the nomogram model. As a result, 5 variables with no signs of multicollinearity (VIF<5) were selected for model construction (Suppl. Table S2). Finally, to assess the probability of NPSLE, a nomogram was constructed (Fig. 5A). For the training set, the AUC of the model to distinguish NPSLE from SLE was 0.914 (95% CI: 0.873–0.955). For the validation set, the AUC of the new model was 0.842(95% CI:0.735–0.950) (Fig. 5B-C). Calibration curves and the Hosmer-Lemeshow test (p>0.05) were used to evaluate the nomogram model (Fig. 5D-E). The results indicated that the model is effective in the training set (χ^2 = 6.412, p=0.601), and validation set (χ^2 =10.578, p=0.227). Excellent consistency between the training and validation sets was observed, indicating high predictive accuracy. Notably, the clinical decision curve demonstrated the model's high safety levels, substantial net benefits, and considerable clinical value (Fig. 5F-G).

Discussion

Diagnosing NPSLE poses a significant challenge due to the absence of a definitive gold standard and the symptoms overlap with other neurological conditions. In our study, we identified TREM2 as a promising biomarker for NPSLE and constructed a novel nomogram model for the probability of developing NPSLE among SLE patients. This study revealed that the nomogram has good discriminatory power, accuracy,

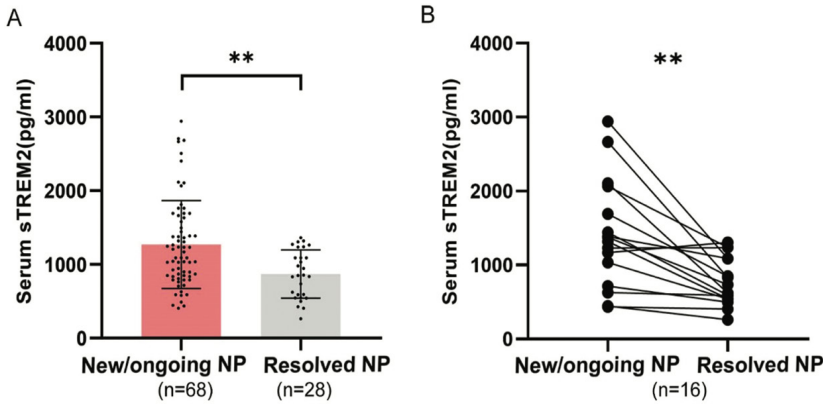

Fig. 4. Serum sTREM2 concentration in SLE patients with new/ongoing and resolved NP events.
A: Serum sTREM2 concentration were significantly lower in SLE patients with resolved events (n=28) compared to those with new/ongoing events (n=68). Statistical analysis was performed using the Mann-Whitney U-test. $^{**}p<0.01$.
B: Serum sTREM2 concentration were significantly decreased after the new/ongoing NP events were resolved (n=16). Statistical analysis was performed using the paired *t*-test. $^{**}p<0.01$.
NP: neuropsychiatric; sTREM2: soluble triggering receptor expressed on myeloid cells 2.

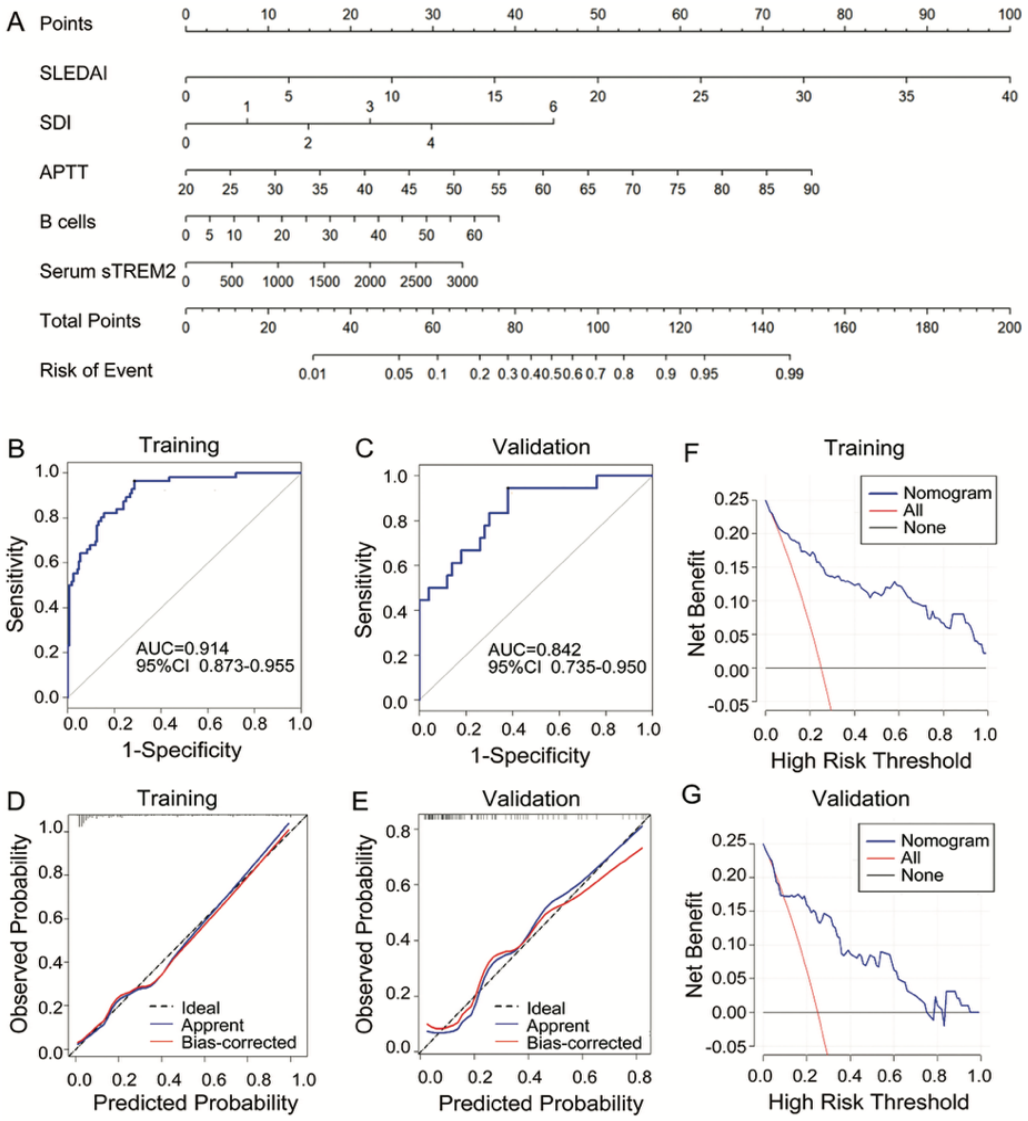

Fig. 5. The construction of the nomogram predictive model and validation of the model.
A: The nomogram for predicting the risk of NPSLE in SLE patients. The overall probability is calculated by taking the sum of the risk points. For each parameter, its risk point can be determined by drawing a vertical line straight up from the parameter's value to the "Points" axis. In order to determine the probability of NPSLE, a vertical line is drawn intersecting the "Total points" with the "Risk of Event" line. **B**: The ROC in the training set. **C**: The ROC in the validation set. **D**: Calibratin curve for training set. **E**: Calibration curve for validation set. **F**: Clinical decision curves for training set; **G**: Clinical decision curves for validation set.
APTT: activated partial thromboplastin time; NPSLE: neuropsychiatric systemic lupus erythematosus; SDI: Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology Damage Index; SLE: systemic lupus erythematosus; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; sTREM2: soluble triggering receptor expressed on myeloid cells 2.

and net clinical benefit based on clinical, and laboratory data (SLEDAI, SDI, APTT, B cells, and serum sTREM2).

TREM2, primarily expressed on microglial cell surfaces, can be cleaved to produce its soluble variant, sTREM2. This soluble form, detectable in both serum and CSF, serves as an indicator of microglial activation (21). Previous studies have reported microglial activation in the brains of NPSLE mouse models, such as NZB/W-F1 lupus-prone mice (22) and MRL/lpr mice (18, 23). Microglial activation has been implicated in the pathogenesis of NPSLE, particularly through synaptic pruning (18, 24). Aligning with these findings, our data demonstrated that TREM2 was a prevalent gene in the upregulated NPSLE pathological pathways, encompassing microglial activation, migration, and synaptic pruning. Our subsequent analysis revealed that serum sTREM2 levels were notably elevated in NPSLE patients – including those with new/ongoing or post-events – compared to non-NPSLE controls (*i.e.* SLE patients without neuropsychiatric events) and healthy individuals. Moreover, we observed elevated CSF sTREM2 levels in NPSLE patients compared to non-NPSLE controls. It is plausible that the enhanced microglial activation in the brains of NPSLE patients contributes to the increased release of sTREM2. In addition, we analysed the serum and CSF levels of sTREM2 in CTD-NP patients. The results showed no significant differences in either serum or CSF sTREM2 levels between CTD-NP patients and other groups, including healthy controls, non-NPSLE controls, and NP-SLE patients. This outcome may be attributed to two main reasons: first, the sample size of the CTD-NP group was too small, with only 6 patients; second, the CTD-NP group included patients with Sjögren's syndrome, dermatomyositis, rheumatoid arthritis, and giant cell arteritis, resulting in significant heterogeneity within the group. Among these, Sjögren's syndrome with neurological involvement shares clinical and pathological features with NPSLE and is often studied together (25, 26). Therefore, microglial activation may also occur in these patients, leading to elevated sTREM2 levels. Indeed, the two Sjögren's syndrome patients in our study exhibited relatively high sTREM2 levels (serum: 1344.64 pg/ml, 859.57 pg/ml; CSF: 854.41 pg/ml, 1210.69 pg/ml). However, due to the small sample size, no significant analysis could be performed. Moreover, we hypothesised that during active NP symptoms, microglial activation would also be more pronounced, leading to increased release of sTREM2. Therefore, we not only analysed all NPSLE patients (including those with new or ongoing NP symptoms as well as those whose symptoms had resolved) but also specifically analysed NPSLE patients with only new or ongoing NP symptoms. However, ROC analysis revealed that the results of these two analyses were similar, with comparable sensitivity (86.25% *vs*. 85.29%) and specificity (57.92% *vs*. 57.35%). This indicates that the diagnostic performance of serum sTREM2 for all NPSLE patients is similar to its performance for diagnosing NPSLE with new or ongoing NP symptoms. In terms of diagnostic precision, our ROC curve analysis indicated that CSF sTREM2 outperformed serum sTREM2 in terms of sensitivity and specificity when distinguishing NPSLE from non-NPSLE controls. Thus, CSF sTREM2 is considered as a potential diagnostic biomarker for NPSLE. However, in clinical settings, obtaining CSF can be challenging; some patients cannot tolerate lumbar punctures. As a result, serum samples, which are more readily accessible, render serum sTREM2 a viable candidate for NPSLE diagnosis.

NPSLE manifests with a broad spectrum of clinical features, encompassing focal neurological syndromes, diffuse psychiatric syndromes – such as cognitive disturbances, mood fluctuations, anxiety disorders, psychosis, and acute confusional state (ACS) – as well as peripheral nervous system (PNS) presentations (1). Among these, ACS stands out as particularly severe, often leading to an unfavourable prognosis (27). In our study, we noted elevated levels of serum sTREM2 in ACS-NPSLE compared to those in patients with non-ACS diffuse NPSLE and focal NPSLE. Intriguingly, differences in CSF sTREM2 levels were not observed among the various NPSLE subgroups. Previous investigations have indicated higher CSF sTREM2 concentrations in ACS-NPSLE relative to non-ACS diffuse NPSLE (28). The divergence in our observations from those of prior research may be attributable to the limited sample size in our study. Nonetheless, our findings regarding serum sTREM2 are noteworthy, especially given the challenges in obtaining CSF in a clinical setting. This suggests a correlation between sTREM2 levels and NPSLE severity.

Distinctly, NPSLE patients can be divided into two primary categories: those presenting with new or ongoing neuropsychiatric (NP) events and those in whom such events have relieved (4). Our analysis, which encompassed patients from both categories, revealed diminished serum sTREM2 levels in NP-SLE patients with resolved NP events in comparison to NPSLE patients with new/ongoing NP events. A paired comparison further corroborated that serum sTREM2 levels markedly decreased in the same NPSLE patient post-resolution of NP events. This underscores the potential association of serum sTREM2 levels with the emergence of NP events. Although we proved that sTREM2 was increased in CSF and serum of NPSLE patients and that serum sTREM2 was associated with the severity and emergence of NP events, the low specificity critically weakens the value of sTREM2 as a diagnostic biomarker for NPSLE. In order to make up for the above disadvantages, we further developed a nomogram model to assess the predictors of NPSLE, which included SLEDAI, SDI, prolonged APTT, percentage of B cells, and serum sTREM2.

Historically, numerous studies have explored predictors predisposing individuals to NPSLE, underscoring the essentiality of these factors in refining diagnostic procedures and tailoring therapeutic interventions. Several studies have shown that higher SLE disease activity is associated with the development of NPSLE (6, 29-32). In line with previous findings, our study suggested that higher SLEDAI is one of the risk factors for developing NPSLE. Previous studies have reported that higher SLEDAI usu-

ally indicated SLE flares and correlated to stronger immune response, including increased production of mediators (such as IFN-γ, IL-10) and enhanced innate immune functions (33, 34). In addition, IFN-γ and IL-10 have been proven to regulate microglia function in NPSLE mouse models and contribute to the pathogenesis of NPSLE (35, 36). Our findings indicate that higher SLE disease activity may promote the development of NPSLE as a result of enhanced immune responses.

Previous studies have not reported the association between SDI and the development of NPSLE. However, there were some studies that identified some organ involvements were risk factors for NPSLE, including shrinking lung syndrome, myalgias/myositis, haemolytic anaemia and cerebrovascular disease (37, 38). First, NP events are capable to contribute to higher SDI directly and patients with the NP manifestations reveal greater damage accrual (38). In addition, we speculated that higher SDI may also be related to enhanced immune responses, which could promote the development of NPSLE.

This study identified APTT as an important predictor of NPSLE. The APTT prolongation in SLE patients is usually caused by the presence of lupus anticoagulant (LA), which is one of the three criteria for antiphospholipid antibodies (aPLs) for the identification of the antiphospholipid syndrome (APS). A number of studies have reported that the positive aPLs or APS were associated with NPSLE (6, 31, 32, 39, 40). However, our study did not show a correlation between aPLs positivity and NPSLE. According to the up-to-date theory of NPSLE pathophysiology, two separate main pathogenic mechanisms lead to NPSLE: 1. Autoimmune or inflammation caused by autoantibodies or inflammatory mediators; 2. Vascular injury and occlusion characterised by a thrombotic process of intracranial vessels due to autoantibody-mediated vascular injury, immune complexes, complement deposition, leukoagglutination, and accelerated atherosclerosis (41). The aPLs positivity or APS promotes the development of ischaemic NPSLE by participating in the thrombotic process of intracranial vessels. The controversy between our study and previous studies may be caused by the different proportions of NPSLE subtypes we included. In addition, previous studies never included coagulation parameters in the analysis of risk factors for NPSLE. Thus, APTT probably is a more specific and sensitive predictor of NPSLE.

B cells exert vital roles in the development of NPSLE. First, autoreactive B cells produce autoantibodies, leading to immune-mediated inflammation and injury. Secondly, B cells are capable to present antigens and activate T cells. Additionally, B cells produce various cytokines and contribute to inflammation and autoimmune responses (42). Our study analysed the association between lymphocyte subpopulations and the development of NPSLE for the first time. Although previous studies have not reported whether lymphocyte subpopulations were related to NPSLE, an excessive number of lymphocytes was considered to inhibit the development of NPSLE via immunosuppression (43). Since the lymphocyte consists of different subpopulations and they play different roles in regulating immune responses, it is reasonable to believe that our results surpass the previous finding and B cells were the main players the promote NPSLE development rather than other lymphocyte subpopulations.

This study identified a novel biomarker sTREM2 and incorporated clinical as well as laboratory parameters into a diagnostic model that predicts the development of NPSLE. We used a nomogram to calculate the risk of NPSLE for each SLE patient. Evaluation and validation of the model, the nomogram showed an excellent performance with good sensitivity and specificity. In the external validation cohort, this prediction model showed good calibration. Also, the depicted DCA showed that intervention decisions based on the predictive model are clearly beneficial.

The limitations of our study deserve some discussion. Foremost, the retrospective design might have introduced biases and potential inaccuracies in data retrieval and interpretation. Further, the sample size of CSF in our study was small. Clinically, procuring CSF from SLE patients without neuropsychiatric symptoms proves challenging, given the absence of a clear indication for lumbar puncture and the fact that some NPSLE patients may not tolerate the procedure. To surmount these limitations, we are keen on forging alliances with peer research groups, aiming to augment our sample repertoire. Concurrently, we aspire to conduct prospective studies, seeking deeper and more definitive insights into sTREM2's clinical implications in NPSLE and further validate the efficacy of our model.

Conclusion

Serum sTREM2 offers promise as a potential biomarker for NPSLE diagnosis. In addition, indices including sTREM2, SLEDAI, SDI, APTT, and B cells may be independent predictors of NPSLE. This model has good predictive performance and might be a valuable tool for diagnosing NPSLE.

Acknowledgments

We thank all authors for their support with patient data collection, sample collection, data analysis, statistical analysis, and valuable suggestions for the article.

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