Utility of autoantibody against an UCH-L1 epitope as a serum diagnostic marker for neuropsychiatric systemic lupus erythematosus

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Abstract

Objective

Neuropsychiatric systemic lupus erythematosus (NPSLE) is one of the most serious complications of systemic lupus erythematosus (SLE), lacking efficient diagnostic biomarkers. Previous studies have shown that anti-ubiquitin carboxyl hydrolase L1(UCH-L1) autoantibody is a promising cerebrospinal fluid (CSF) biomarker for NPSLE diagnosis. The purpose of this study is to explore the serum autoantibodies against different UCH-L1 epitopes and investigate the potential diagnostic value of serum autoantibodies against different UCH-L1 epitopes in NPSLE.

Methods

The epitopes of UCH-L1 protein were predicted in DNAStar software. The serum levels of different UCH-L1 epitope autoantibodies in 40 NPSLE patients, 32 SLE patients without neuropsychiatric symptoms and 21 healthy controls were determined by enzyme-linked immunosorbent assay (ELISA). Data were analysed using Pearson correlation analysis, ROC curve analysis, nonparametric Mann-Whitney test, t-test and χ^2 test.

Results

We screened three candidate epitopes of UCH-L1 protein. The autoantibody against amino acid 58 to 69 of UCH-L1 (UCH^{58-69}) showed highest diagnostic power in distinguishing NPSLE patients from SLE patients without neuropsychiatric symptoms (p=0.0038). The ROC analysis showed that the specificity and sensitivity of anti-UCH^{58-69} were 92.3% and 37.5%, respectively. In addition, increased serum anti-UCH^{58-69} levels were associated with increased SLEDAI, CSF microprotein, CSF leukocyte count, ESR, AnuA, anti-dsDNA, IgG and IgM but with decrease of C3 in SLE patients.

Conclusion

The serum levels of anti-UCH^{58-69} significantly increased in NPSLE patients compared with SLE patients without neuropsychiatric symptoms and were correlated with disease severity. Anti-UCH^{58-69} autoantibody may become a novel serum biomarker for NPSLE non-invasive diagnosis, which might be applicable for NPSLE early screening and diagnosis.

Key words

UCH-L1, anti-UCH-L1, autoantibody, biomarker, neuropsychiatric systemic lupus erythematosus

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Received on September 30, 2021; accepted in revised form on December 6, 2021.

Funding: this work was supported by the National Natural Science Foundation of China (81971520, 81801619, 81771743), Sanming Project of Medicine in Shenzhen (SZSM201612009) and Peking University People's Hospital Scientific Research Development Funds (RDX2019-03).

Competing interests: none declared.

Introduction

Neuropsychiatric systemic lupus erythematosus (NPSLE) is a serious nervous system complication of SLE, with an incidence of 21–95% (1, 2). It is characterised by headache, epilepsy, aseptic meningitis, cranial neuropathy and other mental symptoms, which are associated with lower quality of life, increased utilisation of health care, disability and irreversible organ damage (3, 4). Furthermore, the clinical heterogeneity of neuropsychiatric involvement in SLE patients leads to poor prognosis (5, 6).

Timely accurate diagnosis of NPSLE is limited by the lack of specific biomarkers. Previous studies have confirmed that there is a significant correlation between many autoantibodies and mental symptoms related to NPSLE. For example, antiphospholipid antibody was associated with cerebrovascular diseases and cognitive impairment (7), and anti-NR2 level was related to depression, short-term memory and learning ability (8). Other NPSLE associated biomarkers include IL-6, Tweak, anti-r-RNP and anti-neuronal antibody (9-12). However, none of them is widely accepted as a specific biomarker for the diagnosis of NPSLE in clinical practice. It is still necessary to identify novel biomarkers with higher specificity and sensitivity in NPSLE diagnosis.

Ubiquitin C-terminal hydrolase L1 (UCH-L1) is a deubiquitinase mainly expressed in neurons and neuroendocrine cells, and also in testis and ovary (13). It is the most abundant protein in brain lysates (1–2%), and its mutation and abnormal functions are related to many nervous system diseases (14, 15). At present, UCH-L1 is considered as a serum biomarker of severe traumatic brain injury (TBI) (16). Neurodegenerative diseases are also associated with UCH-L1 gene dysfunction (17).

Our previous study revealed that autoantibodies against UCH-L1 in CSF showed diagnostic value for NPSLE. However, serum UCH-L1 autoantibody did not show diagnostic significance (18). Since the procedure of CSF collection is invasive and difficult to be accepted by some patients, a more convenient and fast serum test is in need for NPSLE screening and early diagnosis. If we can improve the diagnostic efficacy of UCH-L1 antibody in serum, its detection will be more convenient and less invasive than CSF examination, and may be practically applied to early diagnosis and preventive risk screening, which will be helpful to NPSLE treatment. Therefore, in this study, we screened serum autoantibodies against different UCH-L1 epitopes in SLE patients with and without neuropsychiatric involvement, and identified an UCH-L1 epitope whose serum autoantibodies might become a promising diagnostic marker for NPSLE.

Materials and methods

Patients

From March 2019 to July 2021, blood samples were collected from 72 SLE patients from Department of Rheumatology and immunology, Peking University People's Hospital, Beijing, China. All of the SLE patients fulfilled the 1997 ACR criteria for SLE (19), and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was calculated to evaluate the severity of SLE (20). Among them, 32 cases with active neuropsychiatric symptoms were diagnosed as NPSLE by consultation with rheumatologists, neurologists and psychiatrists and fulfilled the 1999 NPSLE ACR nomenclature and case definitions (21). NPSLE patients combined with other connective tissue diseases (including antiphospholipid syndrome) were excluded. The NPSLE patients were categorised as severe NPSLE (sNPSLE, patients fulfilled 2001 Ainiala's revised criteria (22)) and mild NPSLE (mNPSLE, patients with only headache or mild depression, excluded by the 2001 criteria but conformed to the 1999 criteria). The blood sampling was performed before intensive therapy (such as glucocorticoid pulse therapy) for NPSLE. After treatment, we collected blood samples from 9 NPSLE patients again. Twenty-one age- and gender-matched healthy controls (HC) were recruited from the health examination center of the same hospital. The study was approved by the Ethics Committee of Peking University People's Hospital (approval no. 2019PHB007-

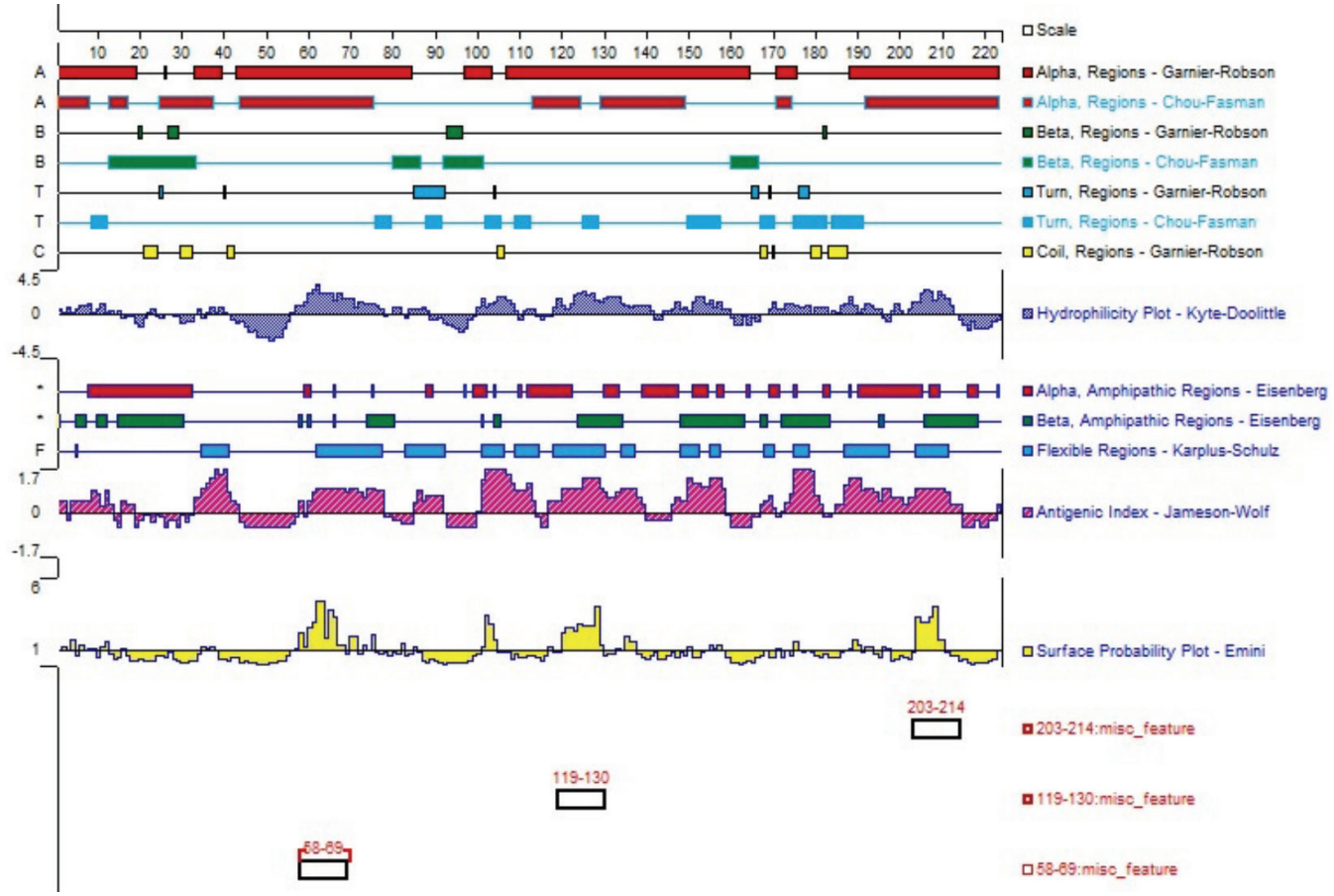


Fig. 1. Prediction results of UCH-L1 epitope peptides. Evaluation of peptides from different antigenic characteristics, such as alpha amphipathic regions (red), beta amphipathic regions (green), hydrophilicity (purple), flexible regions (blue), antigenic index (pink) and surface probability (yellow).

01). All participants of this study provided informed consent for participation in this study.

Screening of potential epitopes of UCH-L1 protein

The epitopes of UCH-L1 protein were predicted according to the statistical tendency of amino acids in DNAStar software. We analysed the protein sequence of UCH-L1 by DNAStar software, including antigenic index of main reference sequence, surface probability of antigen epitope on the original protein surface, and hydrophilicity plot of antigen sequence. Finally, we chose the potential antigenic epitopes by evaluating the following characteristics: alpha amphipathic regions, beta amphipathic regions, hydrophilicity, flexible regions, antigenic index and surface probability.

Clinical and laboratory evaluation

The clinical and laboratory data of SLE patients were recorded, which included age, gender, clinical symptoms, urinalysis, white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), platelet count (PLT), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), anti-nuclear antibodies (ANA), anti-Sm antibody (Sm), anti-ribosomal P protein antibody, anti-SSA antibody (SSA), anti-SSA antibody(SSB), anti-nucleosome antibody (AnuA), anti-double stranded DNA antibody (anti-dsDNA Ab), anti-cardiolipin antibody (aCL), anti-β2-glycoprotein I (β2-GPI), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), complement component 3 (C3) and complement component 4 (C4). SLE disease activity was scored by using the SLE Disease Activity Index (SLEDAI). The NPSLE patients' clinical manifestations and laboratory examinations were also recorded, which included central and peripheral nervous system manifestations, cerebrospinal fluid (CSF) examination results, and radiologic data of the CNS by brain magnetic resonance imaging (MRI) and/or computed tomography (CT) scan.

Leukocytes and platelets less than 4×10^9/L and 10×10^9/L were regarded as leukopenia and thrombocytopenia, respectively. Serum IgA, IgG, IgM, C3, C4, anti-dsDNA Ab, anti-Sm Ab, anti-cardiolipin Ab, and AnuA were determined by ELISA. Complement C3 less than 0.79 g/L and C4 less than 0.16 g/L were considered as decreased C3 and C4, respectively. Moreover, AnuA and anti-dsDNA Ab more than 20RU/ml or 25 IU/ml were regarded as positive, respectively. 24-hour urine excretion more than or equal to 0.5 g/day was defined as proteinuria. Urine leukocytes and erythrocyte more than 14/μl and 24/μl except for urinary tract infection were considered as pyuria and haemoglobinuria, respectively. Urine test including Hb/RBC cylinder or granular cast was defined to be cylindruria. Serum samples were collected with separation gel coagulation tubes (BD, NJ,

USA). All serum samples were frozen at -80 °C until measurement.

Measurement of anti-UCH-L1 epitope autoantibodies

The three synthesised UCH-L1 epitope peptides were purchased from Wuhan Huamei Bioengineering Co., Ltd, Wuhan, China. BSA (Bovine Serum Albumin) was selected as the carrier protein, and the epitope peptides were coupled with BSA. Serum levels of autoantibodies against these epitopes were determined by indirect enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well polysorp plates (Nunc, Denmark) were coated with synthesised UCH-L1 peptides (Huamei, Wuhan, China) of 2μg/mL in carbonate buffer at 4°C overnight. The wells were then washed four times with phosphate-buffered saline containing 0.05% Tween-20 (PBS-T) at room temperature and blocked with 5% albumin bovine V (BSA) for 3 hours at 37°C. After washing with PBS-T for four times, Serum samples were diluted with PBS-T containing 1% BSA at 1:100 and were then added to 96-well plates. After incubation for 1 hour at 37°C, the wells were washed by PBS-T for four times. Then, 100 μL of goat anti-human IgG (Solarbio, Beijing, China) conjugated to peroxidase, diluted at 1:10000, was added to each well and incubated for 40 minutes at 37°C. After washing with PBS-T for four times, Tetramethylbenzidine (Solarbio, Beijing, China) was added as the substrate solution, and five minutes later, the color reaction was stopped by the addition of 50 μl 2M sulfuric acid. Plates were read by a plate reader (BioTek) at an absorbance wavelength of 450 nm optical density (OD 450). The values of OD were transformed to arbitary units (AU), calculated as follows:

$$AU = \frac{[OD_{peptide} - OD_{non\text{-}specific\ background}]_{test\ serum}}{[OD_{peptide} - OD_{non\text{-}specific\ background}]_{positive\ control\ serum}} \times 100$$

Statistical analysis

SPSS21.0 for windows and GraphPad Prism 8 were used to analyse the data. The distribution of numerical data was expressed by the Shapiro-Wilk test. Numerical data with normal distribution and non-normal distribution were presented as mean ± standard deviation and median (range), respectively. Statistical significance between two groups was assessed with the non-parametric Mann-Whitney test, t-test and χ^2 test. Pearson's rank correlation coefficient was applied to calculate the correlations. A *p*-value less than 0.05 was considered to be statistically significant. The cut-off value of levels of the anti-UCH-L1 epitope autoantibodies was determined by receiver operating characteristic (ROC) curve analysis.

Table I Demographic characteristics and neuropsychiatric manifestations of the 32 NPSLE patients.

Type of diseases	Number of patients (%)	Age (years ± SD)
mNPSLE	6/32 (18.8)	35.67 ± 15.67
sNPSLE	26/32 (81.3)	41.73 ± 15.18
SLE controls	40/40 (100)	45.63 ± 17.11
Healthy controls	21/21 (100)	37.33 ± 7.53
Organ involvements, n/N (%)		
Cardiac involvement	3/32 (9.4)	59.00 ± 11.53
Lung involvement	7/32 (21.9)	39.43 ± 12.45
Lupus nephritis	11/32 (34.4)	40.27 ± 15.28
Proteinuria	13/32 (40.6)	39.85 ± 13.74
Haematuresis	8/32 (25)	42.00 ± 17.76
Haematologic involvement	18/32 (56.3)	43.11 ± 16.01
Neuropsychiatric syndromes, n/N (%)		
Aseptic meningitis	1/32 (3.1)	38
Cerebrovascular disease	10/32 (31.3)	47.70 ± 15.99
Movement disorder	1/32 (3.1)	20
Seizure disorders	3/32 (9.4)	38.67 ± 4.73
Psychosis	3/32 (9.4)	33.67 ± 11.06
Acute confusional state	2/32 (6.3)	43.50 ± 0.71
Cognitive dysfunction	1/32 (3.1)	30
Guillain-Barre syndrome	2/32 (6.3)	59.00 ± 1.41
Neuropathy, cranial	2/32 (6.3)	35.00 ± 4.24
Polyneuropathy	7/32 (21.9)	40.57 ± 16.04
Headache	11/32 (34.4)	35.45 ± 13.57
Mood disorder	4/32 (12.5)	50.25 ± 12.18
Cerebrospinal fluid analysis, n/N (%)		
Abnormal appearance	1/20 (5)	29
Intracranial pressure >180 mmH_2O	4/17 (23.5)	34.25 ± 16.19
Intracranial pressure >250 mmH_2O	1/17 (5.9)	18
CSF microprotein (g/L)	1.02 ± 2.65	—
CSF glucose (mmol/L)	3.79 ± 0.89	—
CSF chloride (mmol/L)	124.95 ± 4.17	—
CSF total cell count (cells/uL)	890.95 ± 3857.97	—
CSF leukocyte count (cells/uL)	4.15 ± 15.40	—

mNPSLE: mild neuropsychiatric systemic lupus erythematosus; sNPSLE: severe neuropsychiatric systemic lupus erythematosus; CSF: cerebrospinal fluid. Descriptive statistics for continuous variables were expressed as mean ± SD, and categorical variables were expressed as numbers with percentages.

Results

Screening of UCH-L1 epitopes

We analysed the protein sequence of UCH-L1 by DNAStar software, and screened the potential antigenic epitopes with the following characteristics: alpha amphipathic regions (red), beta amphipathic regions (green), hydrophilicity (purple), flexible regions (blue), antigenic index (pink) and surface probability (yellow) (Fig. 1). Three most suitable peptides were obtained. They were located in the alpha amphiphilic regions, not in the transmembrane region, with high hydrophilicity, high peak value of antigenic index and high surface probability.

The sequence of three UCH-L1 epitope peptides is as follows.

1. 58-69 QHENFRKKQIEE
2. 119-130 SETEKMSPEDRA
3. 203-214 EFTEREQGEVRF

Characteristics of NPSLE patients and controls

We determined the serum levels of autoantibodies against the above three UCH-L1 epitope peptides in 72 SLE

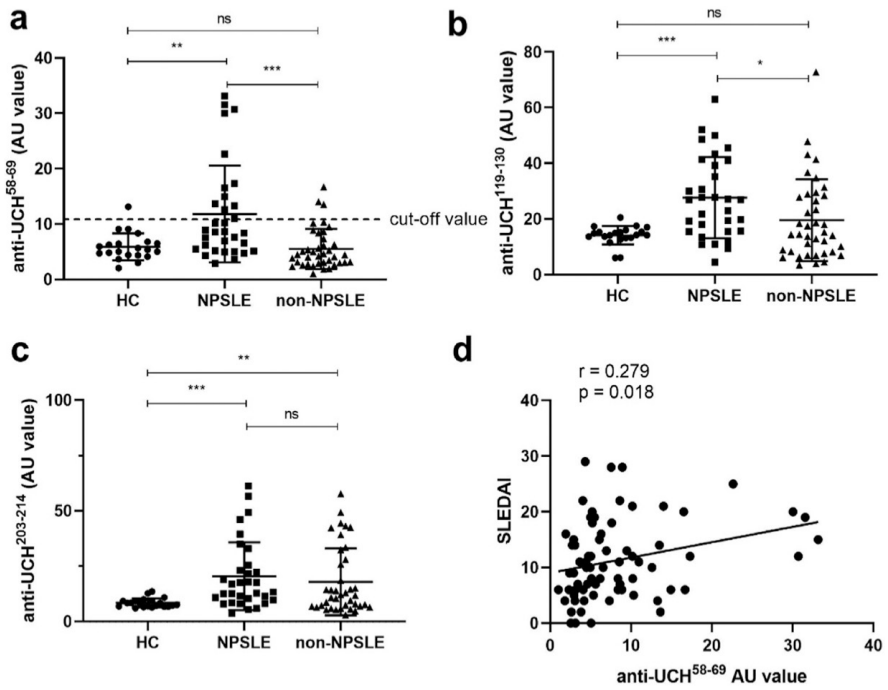


Fig. 2. Serum levels of (**a**) anti-UCH$^{58\text{-}69}$, (**b**) anti-UCH$^{119\text{-}130}$ and (**c**) anti-UCH$^{203\text{-}214}$ autoantibodies in NPSLE, non-NPSLE patients and healthy controls. (**d**) Correlation between anti-UCH$^{58\text{-}69}$ and SLEDAI. *p<0.05, ** p<0.01, ***p<0.001, ns, not significant.

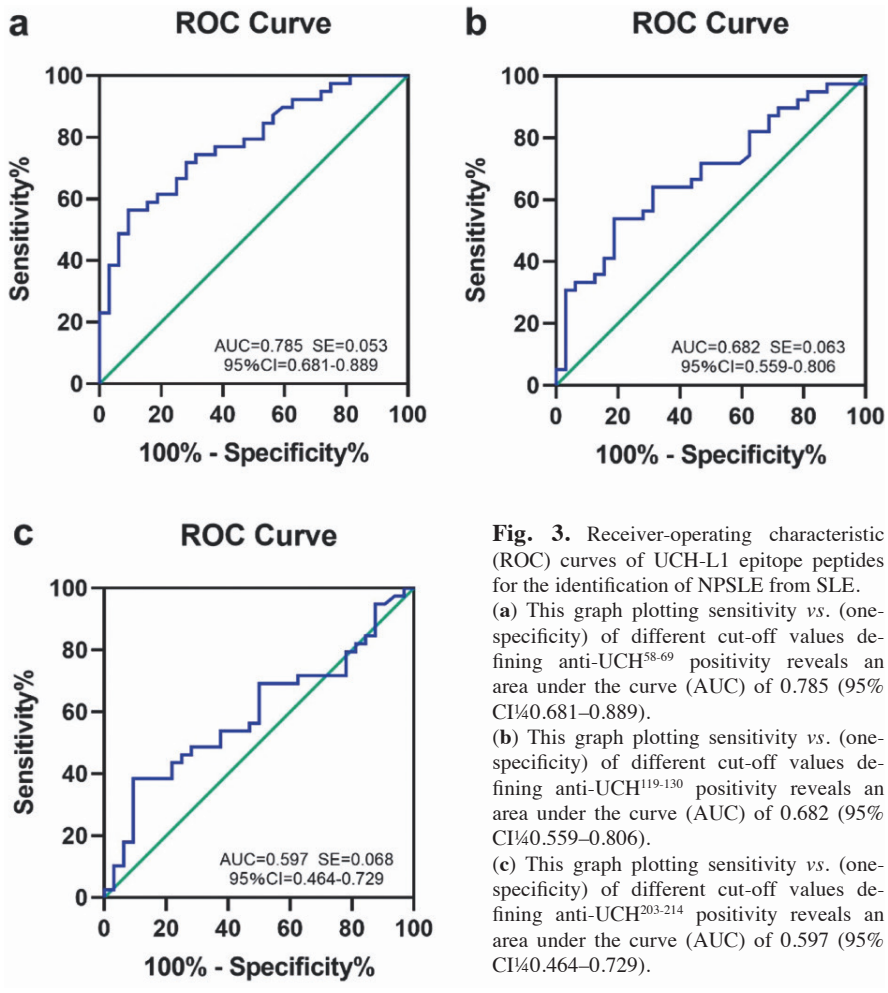


Fig. 3. Receiver-operating characteristic (ROC) curves of UCH-L1 epitope peptides for the identification of NPSLE from SLE.
(**a**) This graph plotting sensitivity *vs.* (one-specificity) of different cut-off values defining anti-UCH$^{58\text{-}69}$ positivity reveals an area under the curve (AUC) of 0.785 (95% CI¼0.681–0.889).
(**b**) This graph plotting sensitivity *vs.* (one-specificity) of different cut-off values defining anti-UCH$^{119\text{-}130}$ positivity reveals an area under the curve (AUC) of 0.682 (95% CI¼0.559–0.806).
(**c**) This graph plotting sensitivity *vs.* (one-specificity) of different cut-off values defining anti-UCH$^{203\text{-}214}$ positivity reveals an area under the curve (AUC) of 0.597 (95% CI¼0.464–0.729).

patients and 21 healthy controls with matched sex and age, then further compared whether there were differences between 32 NPSLE patients and 40 SLE patients without neuropsychiatric symptoms (designated as non-NPSLE patients in the following context). The demographic characteristics and neuropsychiatric manifestations of these patients were shown in Table I. Among the 19 neuropsychiatric manifestations defined by 1999 NPSLE criteria, 12 manifestations were present in the NPSLE patients, and 81.3% (26/32) of them showed two or more overlapped neuropsychiatric manifestations. Apart from four patients with mild depression, all of the mNPSLE patients merely presented with lupus headache (21, 22).

Elevated serum anti-UCH$^{58\text{-}69}$ in NPSLE patients

Compared with the results of the other two peptides, the level of autoantibodies against amino acid 58 to 69 of UCH-L1 (anti-UCH$^{58\text{-}69}$) in NPSLE patients (AU value: 11.82 (2.90–33.16)) was significantly higher than both of non-NPSLE patients (AU value: 5.50 (1.02–16.69)) (p=0.0038) and healthy controls (AU value: 5.88 (2.06–13.11)) (p=0.0001) (Fig. 2a). These results suggested that anti-UCH$^{58\text{-}69}$ in serum might distinguish NPSLE patients from non-NPSLE patients or healthy controls. Although anti-UCH$^{119\text{-}130}$ and anti-UCH$^{203\text{-}214}$were also significantly elevated compared with that of HCs, neither of them could distinguish NPSLE patients from non-NPSLE patients efficiently (Fig. 2b-c).

Diagnostic power of anti-UCH$^{58\text{-}69}$ for NPSLE

To further evaluate the diagnostic power of anti-UCH$^{58\text{-}69}$ for NPSLE, the mean AU value plus two times standard deviation of anti-UCH$^{58\text{-}69}$ in healthy control serum was set up as the cut-off value to identify NPSLE patients from the whole SLE cohort. The cut-off value was 10.66 (Fig. 2a). With this cut-off value, the ROC analysis showed a sensitivity of 37.5% and a specificity of 92.3% of anti-UCH$^{58\text{-}69}$ to distinguish NPSLE patients from non-

Table II. Correlation of serum anti-UCH$^{58-69}$ with clinical and laboratory features of SLE patients.

Laboratory features	Anti-UCH$^{58-69}$	
	Pearson r	*p*-value
Age	0.074	0.540
SLEDAI	**0.279**	**0.018**
24h proteinuria (g)	-0.210	0.124
Serological tests (SLE patients)		
WBC (×10^9/L)	0.040	0.738
RBC (×10^9/L)	-0.133	0.270
Hb (g/L)	-0.114	0.346
PLT (×10^9/L)	-0.073	0.548
ESR (mm/h)	**0.451**	**<0.001**
CRP (mg/dL)	-0.116	0.335
Anti-r-RNP (RU/mL)	0.075	0.543
AnuA (RU/mL)	**0.385**	**0.001**
Anti-dsDNA (IU/mL)	**0.438**	**<0.001**
aCL (U/mL)	0.232	0.059
Anti-β2GPI (RU/mL)	0.114	0.356
IgA (g/L)	0.202	0.090
IgG (g/L)	**0.507**	**<0.001**
IgM (g/L)	**0.331**	**0.005**
C3 (g/L)	**-0.350**	**0.003**
C4 (g/L)	0.165	0.169
Cerebrospinal fluid analysis (NPSLE patients)		
Intracranial pressure (mmH_2O)	0.413	0.088
CSF microprotein (g/L)	**0.454**	**0.039**
CSF glucose (mmol/L)	-0.054	0.816
CSF chloride (mmol/L)	-0.237	0.300
CSF total cell count (cells/uL)	-0.112	0.629
CSF leukocyte count (cells/uL)	**0.452**	**0.040**

SLEDAI: systemic lupus erythematosus disease activity index; WBC: white blood cell; RBC: red blood cell; Hb: haemoglobin; PLT: platelet count; ESR: erythrocyte sedimentation rate; CRP: C-reaction protein; Anti-r-RNP: anti-ribosomal RNA-protein antibody; AnuA: anti-nucleosome antibody; Anti-dsDNA: anti-double-stranded DNA antibody; aCL: anti-cardiolipin antibody; Anti-β2GPI: anti-β2-glycoprotein I antibody; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; C3: complement component 3; C4: complement component 4; CSF: cerebrospinal fluid.

NPSLE patients. The area under curve (AUC) was 0.785, and the 95% confidence interval (CI) was 0.681 to 0.889 (Fig. 3a), while the ROC analysis of anti-UCH$^{119-130}$ showed a sensitivity of 59.4% and a specificity of 64.1% (Fig. 3b, and anti-UCH$^{203-214}$ showed a sensitivity of 59.4% and a specificity of 53.8% (Fig. 3c).

Serum anti-UCH$^{58-69}$ levels were associated with increased SLE disease severity

As shown in Table II, the increase of serum anti-UCH$^{58-69}$ was associated with the increase of SLEDAI in SLE patients (r=0.279, *p*=0.018, Fig. 2d and Table II). Importantly, the increased serum level of anti-UCH$^{58-69}$ in NPSLE patients was correlated with the increase of cerebrospinal fluid microprotein and cerebrospinal fluid leukocyte count (r=0.454, *p*=0.039 and r=0.452, *p*=0.040, Fig. 4g-h and Table II). Meanwhile, the increase of anti-UCH$^{58-69}$ was also significantly correlated with the increase of ESR, AnuA, anti-dsDNA, IgG and IgM, and the decrease of C3 in SLE patients (ESR: r=0.451, *p*<0.001, AnuA: r=0.385, *p*=0.001, anti-dsDNA: r=0.438, *p*<0.001, IgG: r=0.507, *p*<0.001, IgM: r=0.331, *p*=0.005 and C3: r= -0.350, *p*=0.003, Fig. 4a-f and Table II). These results demonstrate that the increase of serum anti-UCH$^{58-69}$ is related to the severity of SLE and the injury of central nervous system.

Anti-UCH$^{58-69}$ is an indicator of sNPSLE

According to the cut-off value, NPSLE patients were divided into anti-UCH$^{58-69}$ positive group (AU value ≥10.66, n=12) and anti-UCH$^{58-69}$ negative group (AU <10.66, n=20). Due to the small number of cases of each NPSLE syndrome, there is no statistical significance of the correlation between anti-UCH$^{58-69}$ and specific NPSLE manifestations. However, the incidence of serious NPSLE manifestations such as cerebrovascular disease and psychosis in the anti-UCH$^{58-69}$ positive group was higher, which proved that anti-UCH$^{58-69}$ might play a role in the development of severe neuropsychiatric manifestations.

It is noteworthy that the intracranial pressure of the patients in anti-UCH$^{58-69}$ positive group were significantly higher than those of anti-UCH$^{58-69}$ negative group (174.00 ± 37.98 *vs*. 112.42 ± 67.05, *p*=0.027), which revealed that anti-UCH$^{58-69}$ may play an important role in the pathogenesis of NPSLE. Further statistical analysis (Table III) showed that ESR, AnuA, Anti-dsDNA, IgG and IgM values of anti-UCH$^{58-69}$ positive group were significantly higher than those of anti-UCH$^{58-69}$ negative group (ESR: 56.00±37.77 *vs*. 22.30±22.07, *p*=0.003; AnuA: 152.97±173.42 *vs*. 22.45±44.55, *p*=0.044; Anti-dsDNA: 114.53±88.32 *vs*. 53.63±66.80, *p*=0.025; IgG: 21.29±6.61 *vs*. 12.48±5.24, *p*=0.001; IgM: 1.70±1.07 *vs*. 0.90±0.46, *p*=0.036). On the contrary, RBC and C3 values were significantly lower than those of anti-UCH$^{58-69}$ negative group (RBC: 3.40±0.94 *vs*. 4.11±0.63, *p*=0.037; C3: 0.46±0.23 *vs*. 0.69±0.21, *p*=0.004). These results suggested SLE patients with increased anti-UCH$^{58-69}$ were more active, and anti-UCH$^{58-69}$ can be used as an indicator of SLE activity.

The influence of treatment on serum anti-UCH$^{58-69}$ levels

The treatment and medication received by all patients are listed in Table IV, and no significant difference was observed between NPSLE patients with and without elevated serum anti-UCH$^{58-69}$. We also compared the levels of serum anti-UCH$^{58-69}$ in 9 NPSLE patients positive for serum anti-UCH$^{58-69}$ at neuropsychiatric symptom onset and remission after treatment. The AU values of anti-UCH$^{58-69}$ decreased significantly after treatment (*p*=0.0162, Fig. 5), which indicated that therapies

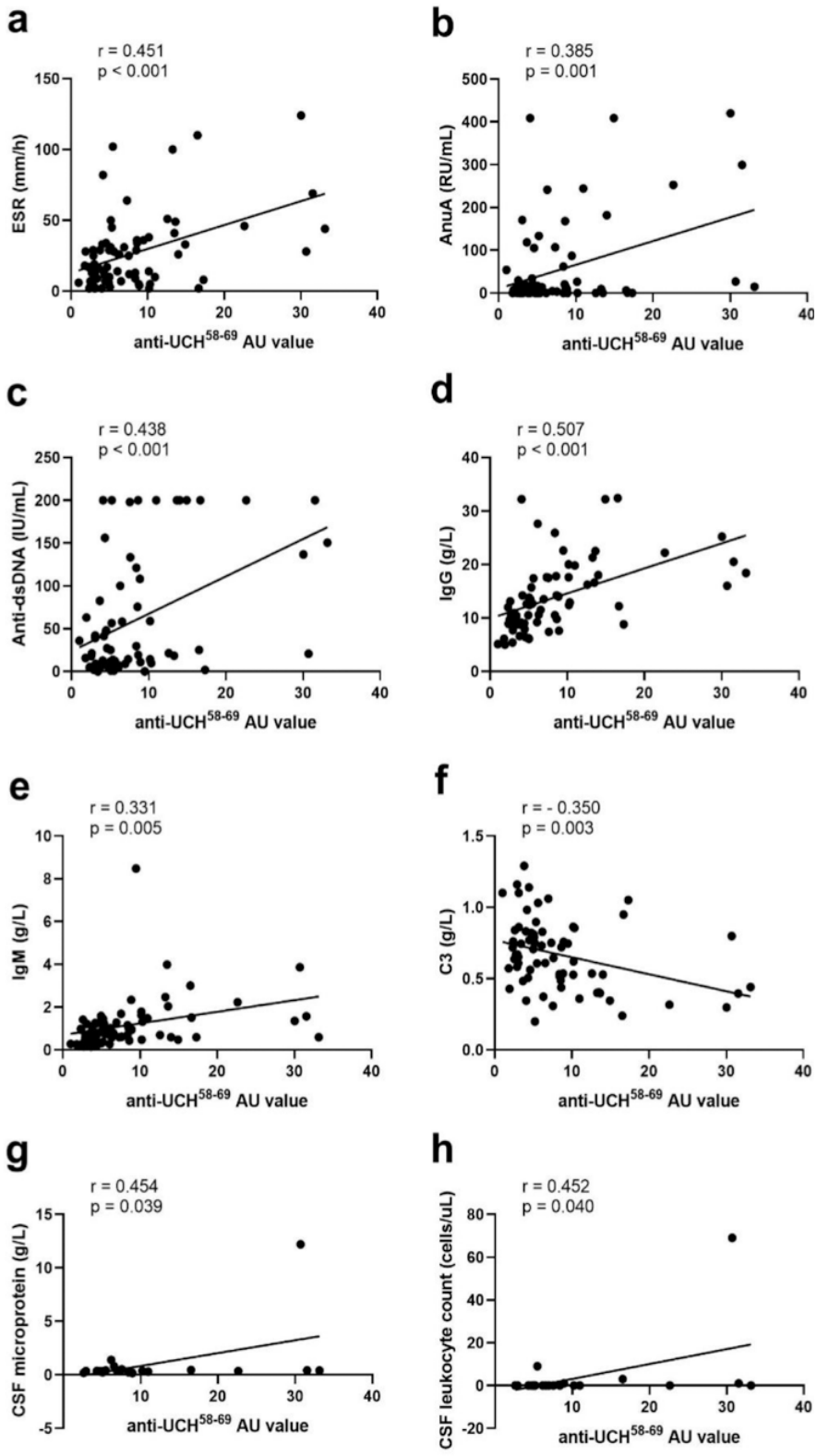


Fig. 4. Pearson's correlation between the serum level of anti-UCH$^{58-69}$ with laboratory parameters in SLE patients.
(**a**) Pearson's correlation between anti-UCH$^{58-69}$ AU value and ESR, (**b**) Pearson's correlation between anti-UCH$^{58-69}$ AU value and AnuA, (**c**) Pearson's correlation between anti-UCH$^{58-69}$ AU value and Anti-dsDNA, (**d**) Pearson's correlation between anti-UCH$^{58-69}$ AU value and IgG, (**e**) Pearson's correlation between anti-UCH$^{58-69}$ AU value and IgM, (**f**) Pearson's correlation between anti-UCH$^{58-69}$ AU value and C3, (**g**) Pearson's correlation between anti-UCH$^{58-69}$ AU value and CSF microprotein, and (**h**) Pearson's correlation between anti-UCH$^{58-69}$ AU value and CSF leukocyte count.

might affect anti-UCH$^{58-69}$ levels in NPSLE patients.

Discussion

It has been reported that UCH-L1 is released from injured neurons, then enters cerebrospinal fluid (CSF) and finally reaches the circulating blood (23), making it widely used as a therapeutic and diagnostic target for nerve injury. A recent study showed that even moderate and mild TBI can easily detect UCH-L1 in serum and plasma within 24 hours after injury (24). Therefore, it is possible that UCH-L1 may be released and induce the production of autoantibodies against UCH-L1 during autoimmune nerve injury. Our previous study has revealed that anti-UCH-L1 is a promising CSF biomarker for NPSLE diagnosis but could not identify NPSLE patients as a serum biomarker (18). However, the method of obtaining CSF is invasive, making the application of CSF in biomarker detection for diagnosis far less convenient and safe than that with serum. Therefore, we screened the specific epitopes of UCH-L1, and confirmed that the serum autoantibodies against one specific epitope of UCH-L1 (anti-UCH$^{58-69}$) may be a promising serum diagnostic marker of NPSLE.

In this study, we explored the diagnostic value of serum anti-UCH$^{58-69}$ autoantibody in differentiating NPSLE patients from SLE patients. The level of anti-UCH$^{58-69}$ was increased in NPSLE, especially compared with other SLE patients and healthy controls, indicating that UCH-L1 induced specific autoimmune response in NPSLE patients. According to our study, anti-UCH$^{58-69}$ has good diagnostic significance (a specificity of 92.3% and a sensitivity of 37.5% to distinguish NPSLE patients from non-NPSLE patients). Our data show that anti-UCH$^{58-69}$ are correlated with SLE disease activity, and not all patients have high titres of anti-UCH$^{58-69}$ antibodies. Only patients with severe conditions have high anti-UCH$^{58-69}$ antibodies, which might affect the nervous system and lead to more severe NPSLE. In contrast, in patients with low disease activity, the anti-UCH$^{58-69}$ levels are lower, so this

Table III. Clinical and laboratory characteristics of NPSLE patients with the elevated and normal levels of serum anti-UCH$^{58-69}$.

Clinical and laboratory characteristics	Anti-UCH$^{58-69}$		t/u/χ^2	*p*-value
	negative	positive		
Age	36.65 ± 13.77	47.17 ± 15.78	-1.909	0.056
SLEDAI	15.10 ± 7.04	13.00 ± 7.06	-0.547	0.584
24h proteinuria (g)	0.45 ± 0.68	0.24 ± 0.13	-0.341	0.733
mNPSLE	4/20 (20)	2/12 (16.7)	0.055	0.815
sNPSLE	16/20 (80)	10/12 (83.3)	0.055	0.815
Organ involvements				
Cardiac involvement	1/20 (5)	2/12 (16.7)	1.202	0.273
Lung involvement	5/20 (25)	2/12 (16.7)	0.305	0.581
Lupus nephritis	7/20 (35)	4/12 (33.3)	0.009	0.923
Proteinuria	9/20 (45)	4/12 (33.3)	0.423	0.515
Haematuresis	5/20 (25)	3/12 (25)	0.000	1.000
Haematologic involvement	11/20 (55)	7/12 (58.3)	0.034	0.854
Neuropsychiatric manifestations				
Aseptic meningitis	1/20 (5)	0/12 (0)	0.619	0.431
Cerebrovascular disease	5/20 (25)	5/12 (41.7)	0.970	0.325
Movement disorder	1/20 (5)	0/12 (0)	0.619	0.431
Seizure disorders	2/20 (10)	1/12 (8.3)	0.025	0.876
Psychosis	1/20 (5)	2/12 (16.7)	1.202	0.273
Acute confusional state	2/20 (10)	0/12 (0)	1.280	0.258
Cognitive dysfunction	1/20 (5)	0/12 (0)	0.619	0.431
Guillain-barre syndrome	2/20 (10)	0/12 (0)	1.280	0.258
Neuropathy, cranial	2/20 (10)	0/12 (0)	1.280	0.258
Polyneuropathy	4/20 (20)	3/12 (25)	0.110	0.740
Headache	9/20 (45)	2/12 (16.7)	2.669	0.102
Mood disorder	3/20 (15)	1/12 (8.3)	0.305	0.581
Serological tests				
WBC (×10^9/L)	6.25 ± 2.89	5.49 ± 2.76	-0.818	0.413
RBC (×10^9/L)	**4.11 ± 0.63**	**3.40 ± 0.94**	**-2.083**	**0.037**
Hb (g/L)	118.30 ± 14.90	103.50 ± 27.41	-1.285	0.199
PLT (×10^9/L)	192.75 ± 57.35	136.67 ± 88.27	-1.733	0.083
ESR (mm/h)	**22.30 ± 22.07**	**56.00 ± 37.77**	**-2.940**	**0.003**
CRP (mg/dL)	3.03 ± 5.03	5.36 ± 5.05	-1.226	0.220
Anti-Sm	3/19 (15.8)	2/11 (18.2)	0.029	0.865
Anti-U1RNP	9/19 (47.4)	5/11 (45.5)	0.010	0.919
Anti-SSA	12/19 (63.2)	7/11 (63.6)	0.001	0.979
Anti-SSB	4/19 (21.1)	1/11 (9.1)	0.718	0.397
ANA (titre)	240.00 ± 236.82	312.73 ± 270.60	-0.950	0.342
Anti-r-RNP (RU/mL)	23.13 ± 58.08	52.34 ± 94.19	-0.732	0.464
AnuA (RU/mL)	**22.45 ± 44.55**	**152.97 ± 173.42**	**-2.014**	**0.044**
Anti-dsDNA (IU/mL)	**53.63 ± 66.80**	**114.53 ± 88.32**	**-2.246**	**0.025**
aCL (U/mL)	6.63 ± 7.96	10.54 ± 9.89	-1.639	0.101
Anti-β2 GPI (RU/mL)	23.40 ± 47.33	17.04 ± 19.58	-0.445	0.657
IgA (g/L)	2.76 ± 1.55	3.08 ± 1.36	-0.876	0.381
IgG (g/L)	**12.48 ± 5.24**	**21.29 ± 6.61**	**-3.446**	**0.001**
IgM (g/L)	**0.90 ± 0.46**	**1.70 ± 1.07**	**-2.102**	**0.036**
C3 (g/L)	**0.69 ± 0.21**	**0.46 ± 0.23**	**-2.842**	**0.004**
C4 (g/L)	2.01 ± 3.32	1.89 ± 2.45	-0.331	0.741
Cerebrospinal fluid analysis				
Intracranial pressure (mmH_2O)	**112.42 ± 67.05**	**174.00 ± 37.98**	**-2.215**	**0.027**
CSF microprotein (g/L)	0.45 ± 0.31	2.36 ± 4.83	-1.156	0.248
CSF glucose (mmol/L)	3.80 ± 1.01	3.77 ± 0.59	-0.330	0.741
CSF chloride (mmol/L)	125.62 ± 3.74	123.38 ± 5.06	-1.361	0.173

SLEDAI: systemic lupus erythematosus disease activity index; mNPSLE: mild neuropsychiatric systemic lupus erythematosus; sNPSLE: severe neuropsychiatric systemic lupus erythematosus; WBC: white blood cell; RBC: red blood cell; Hb: haemoglobin; PLT: platelet count; ESR: erythrocyte sedimentation rate; CRP: C-reaction protein; Anti-Sm: anti-Sm antibody; Anti-r-RNP: anti-ribosomal RNA-protein antibody; Anti-SSA: anti-SSA antibody; Anti-SSB: anti-SSB antibody; ANA: anti-nuclear antibody; Anti-U1RNP: anti-ribonucleoprotein (RNP) antibody; AnuA: anti-nucleosome antibody; Anti-dsDNA: anti-double-stranded DNA antibody; aCL: anti-cardiolipin antibody; Anti-β2GPI: anti-β2-glycoprotein I antibody; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; C3: complement component 3; C4: complement component 4; CSF: cerebrospinal fluid. Descriptive statistics for continuous variables were expressed as mean ± SD and categorical variables were expressed as numbers with percentages.

antibody is positively correlated with disease activity of NPSLE patients. NPSLE patients with lower disease activity do not have high anti-UCH$^{58-69}$ levels and may have negative tests.

The blood sampling performed immediately after the onset of NPSLE, indicating that it would correlate with the disease activity of NPSLE. SLEDAI is an index that is strongly affected by NP symptoms. Among them, NPSLE related scores such as epilepsy, cerebrovascular disease and other mental symptoms account for a large proportion of SLEDAI. If the anti-UCH$^{58-69}$ has a strong correlation with NPSLE, it might also show a strong correlation with SLEDAI. Therefore, using SLEDAI as an index of disease activity for correlation analysis cannot accurately reflect the relationship between anti-UCH$^{58-69}$ and other organ involvement in SLE. In future studies, we will design a prospective study to observe the association between antibodies and organ injury other than neuropsychiatric symptoms of SLE.

We compared the anti-UCH$^{58-69}$ levels by organ involvement. The results showed that there was little correlation between other organ involvement and anti-UCH$^{58-69}$ levels, suggesting that the correlation between anti-UCH$^{58-69}$ and SLEDAI may indeed be due to its close correlation with NPSLE symptoms, and also supporting that the anti-UCH$^{58-69}$ is a specific biomarker of NPSLE. In the anti-UCH$^{58-69}$ positive NPSLE group, RBC and C3 decreased, ESR, IgG and IgM increased, and lupus specific antibodies AnuA and anti-dsDNA increased significantly, especially intracranial pressure increased. These laboratory parameters indicate disease activity in lupus and lupus encephalopathy, indicating that anti-UCH$^{58-69}$ may be helpful to identify NPSLE patients with severe disease activity. Moreover, serum anti-UCH$^{58-69}$ AU values of NPSLE patients significantly decreased after treatment, which suggested that anti-UCH$^{58-69}$ may be used as an indicator to reflect the patient's disease condition and treatment effect. Due to the lack of efficient laboratory diagnostic methods for NPSLE with complex clinical conditions, anti-UCH$^{58-69}$ may

Table IV. Previous therapies in anti-UCH$^{58-69}$-positive and anti-UCH$^{58-69}$-negative patients.

Therapy	anti-UCH$^{58-69}$ + NPSLE, n=12 (%)	anti-UCH$^{58-69}$ - NPSLE, n=20 (%)	t/u/χ^2	*p*-value
GCs	12 (100)	20 (100)	—	—
MTX	2 (16.7)	6 (30)	0.711	0.399
CTX	3 (25)	8 (40)	0.748	0.387
LEF	1 (8.3)	0 (0)	1.720	0.190
MMF	4 (33.3)	10 (50)	0.847	0.358
HCQ	5 (41.7)	12 (60)	1.012	0.314
IVIG	5 (41.7)	3 (15)	2.844	0.092
CYC	2 (16.7)	0 (0)	3.556	0.059
GTW	1 (8.3)	0 (0)	1.720	0.190
Rituximab	1 (8.3)	1 (5)	0.142	0.706

GCs: glucocorticoids; MTX: methotrexate; CTX: cyclophosphamide; LEF: leflunomide; MMF: mycophenolate mofetil; HCQ: hydroxychloroquine; IVIG: intravenous immunoglobulin; CYC: cyclosporine; GTW: Tripterygium wilfordii.

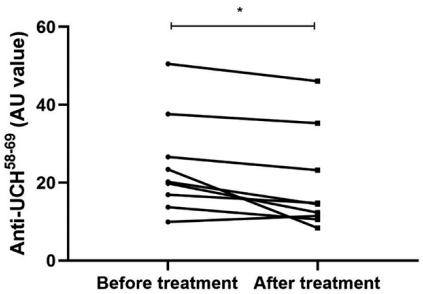
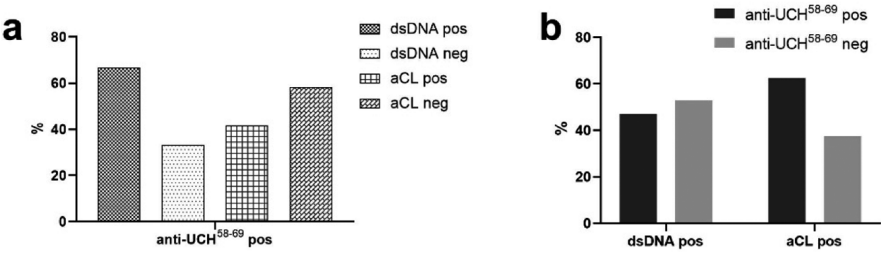


Fig. 5. Serum anti-UCH$^{58-69}$ AU value in anti-UCH$^{58-69}$-positive NPSLE patients before and after treatment.

help clinicians to make a more accurate diagnosis for at least a portion of NPSLE patients.

We observed significant correlation between anti-UCH$^{58-69}$ and anti-dsDNA and a marginally correlation between anti-UCH$^{58-69}$ and aCL. Since anti-dsDNA and aCL were also reported to be meaningful in NPSLE distinguishment (2, 25), possible cross reaction between anti-UCH$^{58-69}$ and these antibodies might lead to its clinical significance in NPSLE diagnosis. SLE associated dsDNA antibodies generally bind to the conserved region of dsDNA skeleton instead of peptide epitopes. Herrmann *et al*. (26) screened two short DNA sequences from the sera of SLE patients and proved that the antibody bound to a DNA fragment with a size of about 6-12 bp. For anti-phospholipid antibodies, the anticardiolipin antibody recognises the negatively charged cardiolipin on platelet and endothelial cell surfaces as the target antigen (27), which is different from anti-UCH-L1$^{58-69}$. In our study, the ELISA kit (Euroimmun, Germany, EA 1621-9601G) for detecting anticardiolipin antibody is coated with purified anticardiolipin from bovine heart, which belongs to different molecular type from the UCH-L1$^{58-69}$ peptide antigen we studied. Therefore, the possibility that UCH-L1$^{58-69}$ might share similar antigenic determinants with dsDNA and phospholipids is low. It is expected that dsDNA and phospholipid antibodies do not have high homology with anti-UCH$^{58-69}$, but further research is still needed to determine whether there is cross reaction in the future. Besides, our study showed that the ability of anti-dsDNA antibodies and aCL to distinguish NPSLE from SLE patients were different from anti-UCH$^{58-69}$. In this study, 7 patients with negative aCL were anti-UCH$^{58-69}$ positive and 4 patients with negative dsDNA were anti-UCH$^{58-69}$ positive, indicating the ability of anti-UCH$^{58-69}$ to detect aCL-negative and dsDNA-negative NPSLE patients. In anti-UCH$^{58-69}$ positive NPSLE patients, the negative rates of dsDNA and aCL are 33.33% and 58.33% (Supplementary Fig. S1(a)), while the positive rates of anti-UCH$^{58-69}$ in dsDNA and aCL positive NPSLE patients are 47.06% and 62.50% (Suppl. Fig. S1(b)), indicating that the distribution patterns of anti-UCH$^{58-69}$, dsDNA and aCL in patients with NPSLE are not totally overlapped. The above comparison suggested that there is no strong cross reaction among anti-UCH$^{58-69}$, dsDNA and aCL.

In SLE patients, when brain complications occur and blood-brain barrier (BBB) is damaged, the increase of anti-UCH$^{58-69}$ autoantibody may target UCH-L1 expressed in nerve cells (28-30). Experimental evidence shows that autoantibodies reacting with brain antigens are the key factors in the pathogenesis of NPSLE (31). Our study showed that the increased level of anti-UCH$^{58-69}$ in NPSLE patients was correlated with the increase of cerebrospinal fluid microprotein and cerebrospinal fluid leukocyte count. More importantly, the intracranial pressure of the patients in anti-UCH$^{58-69}$ elevated group was significantly higher than that of the anti-UCH$^{58-69}$ normal group. This may lead to the destruction of nerve cells and pathological changes of brain tissue, and then increase the level of CSF microprotein and the number of leukocytes. Due to the small number of cases of each NPSLE syndrome, there is no correlation with a specific NPSLE syndrome, but the incidence of NPSLE manifestations is higher in anti-UCH$^{58-69}$ positive patients. Although not statistically significant in this analysis, preparations are being made to expand the sample for further research. The increase of anti-UCH$^{58-69}$ was also significantly correlated with the increase of ESR, AnuA, anti-dsDNA, IgG and IgM, and the decrease of C3 in



Supplementary Fig. S1. Distribution of anti-UCH$^{58-69}$, aCL and dsDNA in NPSLE patients.
(**a**) The distribution of dsDNA and aCL in anti-UCH$^{58-69}$-positive NPSLE patients.
(**b**) The distribution of anti-UCH$^{58-69}$ in dsDNA-positive and aCL-positive NPSLE patients.

SLE patients, suggesting that NPSLE patients with increased anti-UCH$^{58\text{-}69}$ are more active. Further studies may help us to better understand the neuronal damage in NPSLE and the role of UCH-L1 in this process.

There are some limitations in this study. The number of serum samples we collected is limited. Therefore, we need to expand the sample size for verification in future studies, so as to clarify the clinical correlation between UCH-L1 autoantibodies and NPSLE more accurately.

In summary, our study showed that anti-UCH$^{58\text{-}69}$ autoantibody may become a novel serum biomarker for NPSLE non-invasive diagnosis, which is applicable for NPSLE early screening and diagnosis. Anti-UCH$^{58\text{-}69}$ was significantly associated with NPSLE neuropsychiatric symptoms and SLE disease activity, suggesting its potential role in the induction of brain injury. The exact pathogenesis of anti-UCH$^{58\text{-}69}$ in NPSLE is still unclear, which needs further study.

References

1. TSOKOS GC: Systemic lupus erythematosus. *N Engl J Med* 2011; 365: 2110-21. https://doi.org/10.1056/nejmra1100359
2. AHN GY, KIM D, WON S *et al*.: Prevalence, risk factors, and impact on mortality of neuropsychiatric lupus: a prospective, single-center study. *Lupus* 2018; 27: 1338-47. https://doi.org/10.1177/0961203318772021
3. STOJANOVICH L, ZANDMAN-GODDARD G, PAVLOVICH S, SIKANICH N: Psychiatric manifestations in systemic lupus erythematosus. *Autoimmun Rev* 2007; 6: 421-6. https://doi.org/10.1016/j.autrev.2007.02.007
4. FARGETTI S, UGOLINI-LOPES MR, PASOTO SG *et al*.: Short- and long-term outcome of systemic lupus erythematosus peripheral neuropathy: bimodal pattern of onset and treatment response. *J Clin Rheumatol* 2021; 27 (6S): S212-16. https://doi.org/10.1097/rhu.0000000000001201
5. MAGRO-CHECA C, BEAART-VAN DE VOORDE LJ, MIDDELKOOP HA *et al*.: Outcomes of neuropsychiatric events in systemic lupus erythematosus based on clinical phenotypes; prospective data from the Leiden NP SLE cohort. *Lupus* 2017; 26: 543-51. https://doi.org/10.1177/0961203316689145
6. TRENTIN F, ZUCCHI D, SIGNORINI V, ELEFANTE E, BORTOLUZZI A, TANI C: One year in review 2021: systemic lupus erythematosus. *Clin Exp Rheumatol* 2021; 39: 231-41. https://doi.org/10.55563/clinexprheuma tol/7gzsxp
7. JOHNSEN SJ, VALBORGLAND T, GUDLAUGSSON E, BOSTAD L, OMDAL R: Thrombotic microangiopathy and the antiphospholipid syndrome. *Lupus* 2010; 19: 1569-72. https://doi.org/10.1177/0961203310375262
8. TAY SH, FAIRHURST AM, MAK A: Clinical utility of circulating anti-N-methyl-d-aspartate receptor subunits NR2A/B antibody for the diagnosis of neuropsychiatric syndromes in systemic lupus erythematosus and Sjögren's syndrome: An updated meta-analysis. *Autoimmun Rev* 2017; 16: 114-22. https://doi.org/10.1016/j.autrev.2016.12.002
9. STAMPANONI BASSI M, IEZZI E, DRULOVIC J *et al*.: IL-6 in the cerebrospinal fluid signals disease activity in multiple sclerosis. *Front Cell Neurosci* 2020; 14: 120. https://doi.org/10.3389/fncel.2020.00120
10. FRAGOSO-LOYO H, ATISHA-FREGOSO Y, NUÑEZ-ALVAREZ CA, LLORENTE L: Utility of TWEAK to assess neuropsychiatric disease activity in systemic lupus erythematosus. *Lupus* 2016; 25: 364-9. https://doi.org/10.1177/0961203315610206
11. VAN DER MEULEN PM, BARENDREGT AM, CUADRADO E *et al*.: Protein array autoantibody profiles to determine diagnostic markers for neuropsychiatric systemic lupus erythematosus. *Rheumatology* (Oxford) 2017; 56: 1407-16. https://doi.org/10.1093/rheumatology/kex073
12. SATO S, YASHIRO M, ASANO T, KOBAYASHI H, WATANABE H, MIGITA K: Association of anti-triosephosphate isomerase antibodies with aseptic meningitis in patients with neuropsychiatric systemic lupus erythematosus. *Clin Rheumatol* 2017; 36: 1655-9. https://doi.org/10.1007/s10067-017-3653-2
13. TOLICZENKO-BERNATOWICZ, D., MATUSZCZAK E, TYLICKA M *et al*.: Overexpression of ubiquitin carboxyl-terminal hydrolase 1 (UCHL1) in boys with cryptorchidism. *PLoS One* 2018; 13: e0191806. https://doi.org/10.1371/journal.pone.0191806
14. WEATHINGTON NM, MALLAMPALLI RK: Emerging therapies targeting the ubiquitin proteasome system in cancer. *J Clin Invest* 2014; 124: 6-12. https://doi.org/10.1172/jci71 602
15. MATUSZCZAK E, WEREMIJEWICZ A, KOMAROWSKA M *et al*.: Immunoproteasome in the plasma of pediatric patients with moderate and major burns, and its correlation with proteasome and UCHL1 measured by SPR imaging biosensors. *J Burn Care Res* 2018; 39: 948-53. https://doi.org/10.1093/jbcr/iry011
16. SINGH GP, NIGAM R , TOMAR GS *et al*.: Early and rapid detection of UCHL1 in the serum of brain-trauma patients: a novel gold nanoparticle-based method for diagnosing the severity of brain injury. *Analyst* 2018; 143: 3366-73. https://doi.org/10.1039/c8an00533h
17. JARA JH, FRANK DD, OZDINLER PH: Could dysregulation of UPS be a common underlying mechanism for cancer and neurodegeneration? Lessons from UCHL1. *Cell Biochem Biophys* 2013; 67: 45-53. https://doi.org/10.1007/s12013-013-9631-7
18. LI X, SUN J, MU R *et al*.: The clinical significance of ubiquitin carboxyl hydrolase L1 and its autoantibody in neuropsychiatric systemic lupus erythematosus. *Clin Exp Rheumatol* 2019; 37: 474-80.
19. HOCHBERG MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum 1*997; 40: 1725. https://doi.org/10.1002/art.1780400928
20. GLADMAN DD. IBANEZ D, UROWITZ MB: Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002; 29: 288-91.
21. LIANG MH, CORZILLIUS M, BAE SC *et al*.: The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum* 1999; 42: 599-608. https://doi.org/10.1002/1529-0131(199904)42:4%3C599::aid-anr2%3E3.0.co;2-f
22. AINIALA H, HIETAHARJU A, LOUKKOLA J *et al*.: Validity of the new American College of Rheumatology criteria for neuropsychiatric lupus syndromes: a population-based evaluation. *Arthritis Rheum* 2001; 45: 419-23. https://doi.org/10.1002/1529-0131(200110)45:5%3C419::aid-art360%3E3.0.co;2-x
23. LIU MC, AKINYI L, SCHARF D *et al*.: Ubiquitin C-terminal hydrolase-L1 as a biomarker for ischemic and traumatic brain injury in rats. *Eur J Neurosci* 2010; 31: 722-32. https://doi.org/10.1111/j.1460-9568.2010.07097.x
24. POSTI JP, TAKALA RSK, RUNTTI H *et al*.: The levels of glial fibrillary acidic protein and ubiquitin C-terminal hydrolase-L1 during the first week after a traumatic brain injury: correlations with clinical and imaging findings. *Neurosurgery* 2016; 79: 456-64. https://doi.org/10.1227/neu.0000000000001226
25. PRADHAN V, PATWARDHAN M, RAJA DHYAKSHA A, DHAWALE N, GHOSH K: Neuropsychiatric manifestations and associated autoantibodies in systemic lupus erythematosus patients from Western India. *Rheumatol Int* 2015; 35: 541-5. https://doi.org/10.1007/s00296-014-3114-z
26. HERRMANN M, WINKLER TH, FEHR H, KALDEN JR: Preferential recognition of specific DNA motifs by anti-double-stranded DNA autoantibodies. *Eur J Immunol* 1995; 25: 1897-904. https://doi.org/10.1002/eji.1830250716
27. WANG D, LV W, ZHANG S, ZHANG J: Advances in the research on anticardiolipin antibody. *J Immunol Res* 2019; 2019: 8380214. https://doi.org/10.1155/2019/8380214
28. FANOURIAKIS A, BOUMPAS DT, BERTSIAS GK: Pathogenesis and treatment of CNS lupus. *Curr Opin Rheumatol* 2013; 25: 577-83. https://doi.org/10.1097/bor.0b013e328363eaf1
29. DANEMAN R, PRAT A: The blood-brain barrier. *Cold Spring Harb Perspect Biol* 2015; 7: a020412. https://doi.org/10.1101/cshperspect.a020412
30. DUARTE-DELGADO NP, VASQUEZ G, ORTIZ-REYES BL: Blood-brain barrier disruption and neuroinflammation as pathophysiological mechanisms of the diffuse manifestations of neuropsychiatric systemic lupus erythematosus. *Autoimmun Rev* 2019; 18: 426-32. https://doi.org/10.1016/j.autrev.2018.12.004
31. ZANDMAN-GODDARD G, CHAPMAN J, SHOENFELD Y: Autoantibodies involved in neuropsychiatric SLE and antiphospholipid syndrome. *Semin Arthritis Rheum* 2007; 36: 297-315. https://doi.org/10.1016/j.semarth rit.2006.11.003