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Original article

Protein array autoantibody profiles to determine diagnostic markers for neuropsychiatric systemic lupus erythematosus

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Abstract

Objective. The aim was to investigate the association between autoantibodies (autoAbs) and neuropsychiatric (NP) involvement in patients with SLE and to evaluate whether any autoAb or a combination of these autoAbs could indicate the underlying pathogenic process.

Methods. Using a multiplexed protein array for 94 antigens, we compared the serum autoAb profiles of 69 NPSLE patients, 203 SLE patients without NP involvement (non-NPSLE) and 51 healthy controls. Furthermore, we compared the profiles of NPSLE patients with clinical inflammatory (n = 38) and ischaemic (n = 31) NP involvement.

Results. In total, 75 IgG and 47 IgM autoAbs were associated with SLE patients in comparison with healthy controls. Comparing NPSLE with non-NPSLE and healthy control sera, 9 IgG (amyloid, cardiolipin, glycoprotein 2, glycoprotein 210, heparin, heparan sulphate, histone H2A, prothrombin protein and vimentin) and 12 IgM (amyloid, cardiolipin, centromere protein A, collagen II, histones H2A and H2B, heparan sulphate, heparin, mitochondrial 2, nuclear Mi-2, nucleoporin 62 and vimentin) autoAbs were present at significantly different levels in NPSLE. The combination of IgG autoAbs against heparan sulphate, histone H2B and vimentin could differentiate NPSLE from non-NPSLE (area under the curve 0.845, 99.97% CI: 0.756, 0.933; $P < 0.0001$). Compared with non-NPSLE, four IgG and seven IgM autoAbs were significantly associated with inflammatory NPSLE. In ischaemic NPSLE, three IgG and three IgM autoAbs were significantly different from non-NPSLE patients.

Conclusion. In our cohort, the presence of high levels of anti-heparan sulphate and anti-histone H2B combined with low levels of anti-vimentin IgG autoAbs is highly suggestive of NPSLE. These results need to be validated in external cohorts.

Key words: neuropsychiatric lupus erythematosus, systemic lupus erythematosus, autoantigen microarrays, autoantibodies, biomarkers

BASIC SCIENCE

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Rheumatology key messages

- Fifteen autoantibodies were significantly different in NPSLE patients *vs* non-NPSLE patients.
- A diagnostic model with heparan sulphate, vimentin and H2B autoantibodies differentiated NPSLE from non-NPSLE.

Introduction

SLE is a severe chronic autoimmune disease affecting multiple organ systems [1]. Neuropsychiatric (NP) events are common in SLE; however, in only one-third of cases can symptoms be attributed directly to nervous system damage because of SLE, then being referred to as NPSLE [2–5]. The ACR published an NPSLE nomenclature including 12 CNS and 7 peripheral nervous system manifestations [6]. This nomenclature is widely used to create more homogeneous patient groups to facilitate research, but to date there is no formal classification system to establish the diagnosis of NPSLE, nor guidelines regarding therapeutic decisions in these patients. Within the NPSLE patients, two phenotypes are recognized based on potential pathological mechanisms: inflammatory NPSLE, related to a pro-inflammatory and/or autoimmune-mediated cause; and ischaemic NPSLE, associated with vascular occlusion, microangiopathy and haemorrhage [7]. In daily practice, a per-patient multidisciplinary diagnostic and therapeutic approach is recommended, based on the suspected pathological mechanism of NP complaints and severity of clinical symptoms.

The pathophysiology of NPSLE is diverse and complex; many different mechanisms have been proposed to contribute to the nervous system damage in SLE. Despite considerable advances in our understanding of the disease, it is currently not possible to provide a full definition of the pathogenesis of NPSLE, and more importantly, no single complementary test can specifically discriminate between SLE-related and unrelated NP manifestations [2, 5]. In SLE, >100 different autoantibodies (autoAbs) have been described in former research. Thus, it is reasonable to hypothesize that (i) autoAbs may also be implicated in the damage to the nervous system and/or (ii) damaged CNS structures may induce autoAb development. Although several autoAbs in cerebrospinal fluid or serum were shown to be associated with NPSLE [8], a recent meta-analysis showed that no single biomarker was reliably associated with all NP manifestations in SLE patients. In a consecutive sub-analysis, the presence of lupus anticoagulant seemed to be related specifically to cerebrovascular events in SLE [9, 10].

New and large-scale immunoproteomic approaches give the opportunity to study a wide spectrum of autoAbs in NPSLE patients in a more comprehensive way, with higher sensitivity and broader dynamic range than in traditional ELISA systems [11]. Knowledge about the autoAbs implicated in NPSLE could shed light onto potential pathogenesis of nervous system damage in SLE and help to improve diagnosis and treatment in NPSLE. This study aimed at the analysis of associations between autoAbs and the occurrence of NPSLE, assessment of the predictive value of an autoAb profile as a diagnostic marker for NPSLE, and comparison of autoAbs in NPSLE patients with the abovementioned distinct inflammatory or ischaemic phenotypes.

Methods

In total, 272 SLE patients and 51 healthy controls were included in this prospective cross-sectional cohort study. All patients were followed up at Leiden University Medical Center, Leiden, The Netherlands, VU University Medical Center, Amsterdam, The Netherlands or the Academic Medical Center, Amsterdam, The Netherlands. Patients were assessed by a rheumatologist who established the diagnosis of SLE and classified according to the revised 1997 ACR criteria [12]. SLE patients with NP events had a 1-day admission to the Leiden University Medical Centre, a tertiary national referral centre for NPSLE in The Netherlands. During this admission, all patients were evaluated in a standardized multidisciplinary manner by specialists in rheumatology, neurology, psychiatry and vascular medicine. They also underwent extensive neuropsychological testing, serological examination and 3.0 T MRI of the brain. Furthermore, a lumbar puncture, MRI of the spine and magnetic resonance angiography were performed if deemed necessary. Six to 12 months after the admission, all patients were re-evaluated. Finally, establishment of an NPSLE diagnosis using the 1999 ACR case definitions and its distinction into an inflammatory phenotype [clinical suspicion of an autoimmune-mediated cause after exclusion of other aetiologies, supported by the presence of other concomitant organ SLE manifestation or signs of active SLE disease (i.e. complement consumption) and a normal MRI or diffuse cortical grey matter or white matter lesions on MRI] or an ischaemic phenotype (infarctions on MRI of the brain, or thrombotic lesions of brain arteries or venous sinus thrombosis on magnetic resonance angiography, with symptoms matching the location of the lesions, without other aetiology) was based on expert opinions of the multidisciplinary consensus group [6]. A more detailed description of this diagnostic approach is given in the article by Zirkzee *et al.* [7]. Demographic and clinical data at NPSLE onset and treatment regimens at the moment of blood sampling were collected. In addition to the sera of SLE patients, sera from 51 healthy blood donors were collected as controls.

This study was conducted in accordance with the Declaration of Helsinki, and the institutional review boards of the participating centres (Leiden University Medical Center, Leiden, The Netherlands, VU University Medical Centre, Amsterdam, The Netherlands and the Academic Medical Center, Amsterdam, The Netherlands) approved the conduct of the study. Written informed consent was obtained from all patients at the local centres.

Serum collection and manufacture of autoantigen microarrays

Serum samples of SLE patients were obtained during standard blood sampling for clinical care in one of three academic hospitals. In SLE patients with NP events, blood sampling was performed during the diagnostic approach as described above. Sera of all study subjects were aliquoted and stored at −80 °C. Autoantigen microarrays were manufactured in the microarray core facility of University of Texas Southwestern Medical Center, Dallas, TX, USA. A selection of 94 autoantigens was made (supplementary Fig. S1, available at *Rheumatology* Online), based on their implication in various human autoimmune diseases, including SLE, RA, multiple sclerosis and SS [13]. Four controls (IgG, IgM, anti-human IgG and IgM) were also imprinted on the arrays. For protein array analysis, 5 μl serum was pretreated with DNAse-I (50 U/ml) for 30 min at room temperature and then diluted 1:50 with PBS (137 mM NaCl, 9 mM KOH and 11.3 mM NaH_2PO_4, pH 7.0). Then, autoantigens were printed in duplicate to the nitrocellulose-coated 16-pad FAST slides using a GeSim Nanoplotter NP2.1 microarrayer (GeSim, Radeberg, Germany). Total human IgG and IgM were used as internal controls and for normalization of the autoAb reactivity. The slides were kept at 70% humidity for 4 h at room temperature, before storage at 4 °C for up to 6 months without loss of activity.

Hybridization and scanning

Before hybridization, slides were brought to room temperature for 30 min and a 16-sectional frame was administrated to separate the individual arrays. After adding 50 μl blocking buffer (1% BSA in PBS with 0.1% Tween), the arrays were agitated for 30 min at room temperature.

The pretreated serum samples were diluted 1:100 in blocking buffer, which was found to have the optimal signal-to-noise ratio for the majority of targets. Addition of 50 μl of this dilution to each array was followed by incubation for 60 min with agitation. After washing three times with 100 μl PBS (0.1% v/v Tween 20, pH 7.4) and agitation for 5 min, Cy3-labelled anti-human IgG and Cy5-labelled anti-human IgM autoAbs at 1:1000 dilution were applied to each array, followed by a 60 min incubation with agitation. One array on each slide was used as a negative control, with PBS instead of serum. After washing with PBS with 0.1% Tween as before, the slides were immersed in PBS for 1 min and then spun dry. The autoAb reactivity was detected with a GenePix 4000B scanner with laser wavelengths 532 nm (Cy3 for IgG) and 635 nm (Cy5 for IgM). The images were analysed using Genepix Pro 7.0 software (Molecular Devices, Sunnyvale, California, USA). The net fluorescent intensity (NFI) of each antigen was generated by subtracting the local background and negative control signal. The signal-to-noise ratio was also generated for each antigen. The antibody signal was considered significantly higher than background at a signal-to-noise ratio $\geqslant 3$.

Statistical analysis

For statistical analysis, SPSS was used (IBM SPSS Statistics for Windows, version 20.0; IBM Corp., Armonk, NY, USA). All group comparisons were performed with a non-parametric Mann–Whitney *U*-test and a χ^2 test for continuous and categorical data, respectively. Values of $P < 0.05$ were regarded as statistically significant. A Bonferroni correction was used to account for multiple testing; hence, in analysing the patient characteristics, a threshold of $P < 0.0028$ [correcting for the number of patient characteristics (18): 0.05/18] was considered as statistically significant. A Monte Carlo test with 10.000 permutations and a threshold of $P < 0.0003$ [correcting for the number of tested autoAbs (188): 0.05/188] was used to study the robustness of the data set. In analysing the autoAbs between different subgroups, again a threshold of $P < 0.0003$ (0.05/188) was used.

Correlation between the IgM and IgG autoAbs was analysed with a Spearman correlation test using a threshold of $P < 0.0033$ [correcting for the number of tested autoAbs in the correlation analysis (15): 0.05/15]. Correlation was considered weak if the correlation coefficient r was <0.30, moderate with $r \geqslant 0.30$ and <0.50, strong with $r \geqslant 0.50$ and <0.70 and very strong with $r \geqslant 0.70$–1.00. When IgM and IgG autoAbs were significantly correlated, IgG autoAbs were evaluated for their predictive value for NPSLE using a univariate logistic regression and a receiver operating characteristic curve. This analysis also determined optimal cut-off values for dichotomizing the values of autoAb reactivity (in NFI). After this preselection, a profile of dichotomized IgG autoAbs was identified, applying multivariate logistic regression with backward elimination based on the likelihood ratio. Values of $P > 0.10$ and $P < 0.05$ were used as removal and reintroduction criteria, respectively.

A selection of these dichotomized autoAbs were captured in a diagnostic scoring system based on their performance in a multivariate logistic regression with backward elimination based on likelihood ratio, in accordance to a method previously described in an unrelated autoimmune disease process [14]. Weighted scores were assigned by rounding the regression coefficients to the nearest 0.0 or 0.5. Finally, a receiver operating characteristic curve of this model was plotted to evaluate the discriminative performance of the NPSLE diagnostic scoring system.

Heatmaps displaying the reactivity of autoAbs were created using Genesis (version 1.7.6; TU Graz, Austria). Other images were built in GraphPad Prism (version 6.0; GraphPad Software, San Diego, CA, USA).

Results

Patients

We included 51 healthy controls and 272 SLE patients, of whom 69 patients were diagnosed with NPSLE using the 1999 ACR case definitions system. SLE patients without NP manifestations were classified as non-NPSLE (n = 203).

Concerning demographic and clinical characteristics, SLE patients were older and predominantly female when compared with healthy serum controls (supplementary Table S1, available at *Rheumatology* Online). However, no significant differences between NPSLE and non-NPSLE patients in demographic and clinical characteristics were observed (Table 1).

NPSLE *vs* non-NPSLE

Comparing the serum autoAb profiles of all SLE patients as a single cohort with the binding profiles of healthy controls, 75 IgG autoAbs and 47 IgM autoAbs showed significantly higher levels in SLE sera (supplementary Tables S2 and S3, available at *Rheumatology* Online).

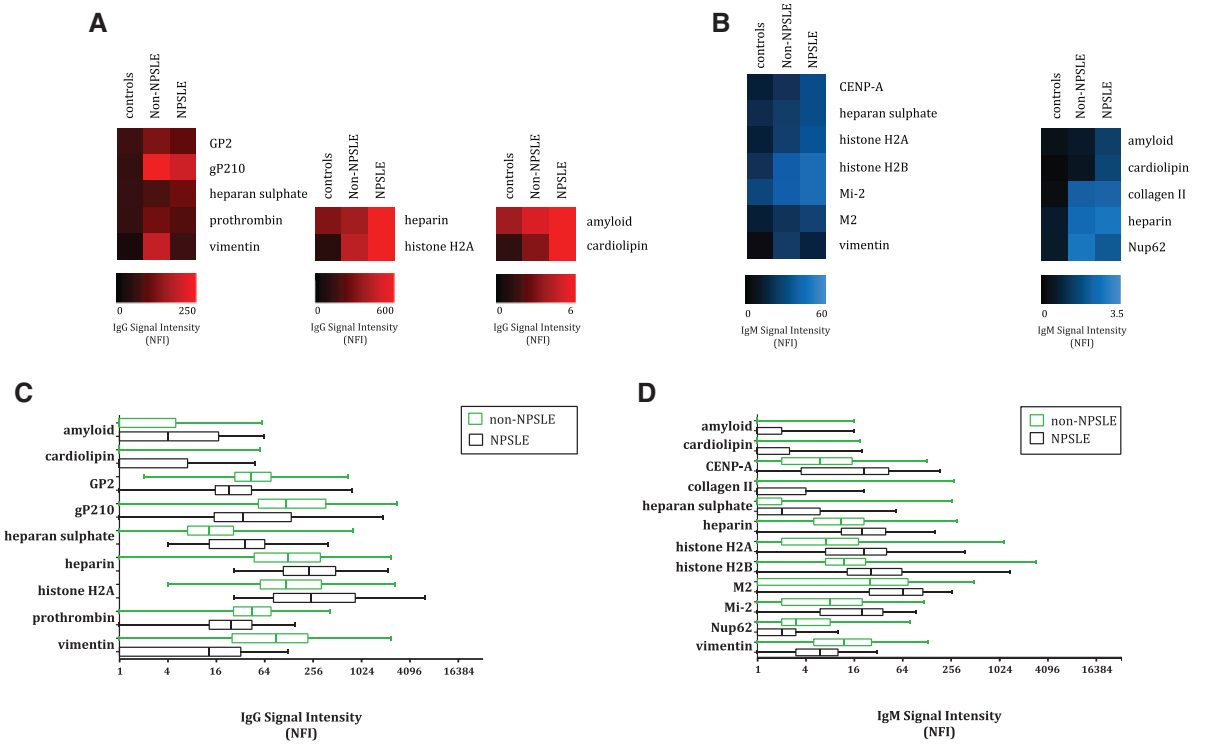
When focusing on the IgG autoAbs that could discriminate NPSLE from non-NPSLE patients, a significantly higher level of IgG autoAbs against five antigens (amyloid, cardiolipin, heparan sulphate, heparin and histone H2A) and significantly lower level of IgG autoAbs against four other antigens (glycoprotein 2 (GP2), glycoprotein 210 (gp210), prothrombin and vimentin) were detected in NPSLE sera (Fig. 1A and C). With respect to IgM autoAbs, a significantly higher level of IgM autoAbs against 10 antigens (amyloid, cardiolipin, CENP-A, collagen II, heparan sulphate, heparin, histone H2A and H2B, nuclear Mi-2 (Mi-2) and mitochondrial 2 (M2)) and a lower level of IgM autoAbs against two antigens (nucleoporin 62 (Nup62) and vimentin) were present in NPSLE sera when compared with non-NPSLE sera

Table 1 Demographic and clinical characteristics of the study cohort, divided into NPSLE and non-NPSLE patients

Patient characteristics	NPSLE	Non-NPSLE	P-value
Number of patients	69	203	–
Age at inclusion, median (IQR), years	42.0 (28.5–49.0)	40.0 (32.0–50.0)	0.632
Age at onset, median (IQR), years	33.0 (21.5–42.0)	31.0 (23.0–42.0)	0.920
Time between SLE onset and NP symptoms, mean (S.D.),[a] years	5.0 (7.3)	N/A	–
Females, n (%)	60 (87.0)	182 (89.7)	0.536
Ethnicity, n (%)			
Caucasian	49 (71.0)	148 (72.9)	0.761
Negroid	3 (4.3)	16 (7.9)	0.320
Asian	13 (18.8)	17 (8.4)	0.017
Arabic	0 (0.0)	15 (7.4)	0.020
Indian/Pakistani	0 (0.0)	3 (1.5)	0.574
Mixed	4 (5.8)	3 (1.5)	0.078
Unknown	0 (0.0)	1 (0.5)	0.559
Medication use, n (%)			
Prednisone	40 (58.0)	111 (54.7)	0.635
Methylprednisolone	12 (17.4)	30 (14.8)	0.604
CYC	9 (13.0)	22 (10.8)	0.618
Anti-CD20	1 (1.4)	9 (4.4)	0.255
ACR case definitions for NPSLE, n (%)			
Cerebrovascular disease	41 (59.4)	N/A	–
Demyelinating syndrome	1 (1.4)	N/A	–
Headache	10 (14.5)	N/A	–
Movement disorder	2 (2.9)	N/A	–
Myelopathy	2 (2.9)	N/A	–
Seizure disorders	10 (14.5)	N/A	–
Acute confusional state	3 (4.3)	N/A	–
Anxiety disorder	4 (5.8)	N/A	–
Cognitive dysfunction	30 (43.5)	N/A	–
Mood disorder	13 (18.8)	N/A	–
Psychosis	6 (8.7)	N/A	–
Aseptic meningitis	0 (0)	N/A	–
ACR case definitions for NPSLE, peripheral nervous system, n (%)			
Autonomic disorder	1 (1.4)	N/A	–
Mononeuropathy	1 (1.4)	N/A	–
Neuropathy, cranial	1 (1.4)	N/A	–
Polyneuropathy	9 (13.0)	N/A	–
Guillain–Barré syndrome	0 (0)	N/A	–
Plexopathy	0 (0)	N/A	–
Myasthenia gravis	0 (0)	N/A	–

Data were statistically compared with Mann–Whitney *U*-tests or χ^2 tests; the associated P-values are listed. [a]These patients represent a mixture of NPSLE patients with NP symptoms at the initial presentation, NP symptoms during follow-up of the disease or a recurrent NP episode. IQR: interquartile range; N/A: not applicable; NP: neuropsychiatric.

Fig. 1 Immunoreactivity in NPSLE *vs* non-NPSLE



Autoantibodies with a statistically significant difference in median NFI value between NPSLE and non-NPSLE patients. (**A**) Heatmap of IgG autoAbs, subdivided as NFI 0–250, NFI 0–600 and NFI 0–6. (**B**) Heatmap of IgM autoAbs, subdivided as NFI 0–60 and NFI 0–3.5. (**C**) Boxplot of IgG autoAbs. Whiskers: from minimal NFI value to maximal NFI value; box = 25–75th percentile; line in box = median; *x*-axis is logarithmic. (**D**) Boxplot of IgM autoAbs. Whiskers: from minimal NFI value to maximal NFI value; box = 25–75th percentile; line in box = median; *x*-axis is logarithmic. AutoAbs: autoantibodies; NFI: net fluorescent intensity.

(Fig. 1B and D). Noticeably, in all cases the IgG and IgM autoAb levels were significantly higher in NPSLE sera when compared with sera from healthy controls (Fig. 1A and B).

Concerning the correlation between IgG and IgM autoAbs, all abovementioned autoAbs except those against gP210, Mi-2 and M2 were significantly correlated with predominantly moderate-to-strong correlation coefficients (supplementary Table S4, available at *Rheumatology* Online).

With regard to the robustness of the data set, all IgG and IgM autoAbs that could discriminate both SLE *vs* healthy controls and NPSLE *vs* non-NPSLE were verified and met significance in Monte Carlo robustness testing.

Inflammatory and ischaemic NPSLE

The 69 NPSLE patients were categorized as 38 inflammatory and 31 ischaemic NP patients. Sera of each phenotype were compared with sera of non-NPSLE patients. In patients with inflammatory NPSLE, a significantly higher level of IgG autoAbs against cardiolipin and heparan sulphate and a lower level of IgG autoAbs against G2P and vimentin were detected (supplementary Fig. S2, available at *Rheumatology* Online). In ischaemic NPSLE, a significantly higher level of IgG autoAbs against heparan sulphate and a lower level of IgG autoAbs against gP210 and vimentin were present (supplementary Fig. S2, available at *Rheumatology* Online).

With regard to IgM immunoreactivity in inflammatory NPSLE, a higher level of autoAbs against amyloid, cardiolipin, heparan sulphate, histones H2A and H2B and a significantly lower level of autoAbs against Nup62 and vimentin were present (supplementary Fig. S3, available at *Rheumatology* Online). In ischaemic NPSLE, a significantly higher level of IgM autoAbs against collagen II was detected, next to heparan sulphate and histone H2A (supplementary Fig. S3, available at *Rheumatology* Online).

However, when comparing inflammatory and ischaemic NPSLE sera, the level of autoAbs in sera was not significantly different.

Autoantibodies as a diagnostic marker for NPSLE

As described, NPSLE and non-NPSLE patients could be distinguished from each other by significantly different values of 9 IgG autoAbs and 12 IgM autoAbs. These autoAbs were directed against 15 antigens in total. As

TABLE 2 Distinguishing NPSLE from non-NPSLE: diagnostic performance of statistically significant different autoantibodies between NPSLE and non-NPSLE

IgG autoantibody	AUC	P-value (of AUC)	Cut-off (NFI)	Sensitivity	Specificity	OR (99.97% CI)
Vimentin	0.807	<0.0001	40.5	88.4	66.0	13.53 (3.34, 54.90)
Heparan sulphate	0.722	<0.0001	20.5	65.2	70.4	4.47 (1.53, 13.02)
Nup62	0.722	<0.0001	26.5	81.2	55.2	4.49 (1.48, 13.59)
Prothrombin	0.686	<0.0001	32.5	65.2	65.5	3.56 (1.24, 10.27)
GP2	0.682	<0.0001	34.5	68.1	65.0	3.97 (1.36, 11.64)
Cardiolipin	0.660	0.0001	0.5	44.9	86.7	5.32 (1.68, 16.81)
Histone H2A	0.653	0.0001	189.0	60.9	64.0	2.65 (0.93, 7.52)
Histone H2B	0.650	0.0002	146.5	63.8	62.6	2.94 (1.03, 8.37)
Collagen II	0.650	0.0002	4.5	65.2	58.1	2.60 (0.91, 7.43)
Heparin	0.647	0.0003	174.0	65.2	61.1	2.77 (0.97, 7.91)
Amyloid	0.647	0.0003	1.5	69.6	58.1	3.17 (1.08, 9.31)
CENP-A	0.524	0.5476	104.5	46.4	63.5	1.19 (0.36, 4.00)

AUC: area under the curve; CENp-A: centromere protein A; NFI: net fluorescent intensity; OR: odds ratio.

TABLE 3 Selected IgG autoantibodies for the NPSLE diagnostic scoring system

IgG autoantibody	Cut-off value	Score if < cut-off	Score if > cut-off
Vimentin	40.5	2.50	0
Heparan sulphate	26.5	0	1.00
Histone H2B	146.5	0	0.50

Cut-off values of the autoantibodies and the corresponding scores associated with the cut-off values. The maximal score that could be attained was 4.0.

TABLE 4 Performance of the NPSLE diagnostic scoring system

Score	Sensitivity (%)	Specificity (%)	Positive predictive value (%)
⩾0	100	0	25
⩾0.5	100	38	36
⩾1.0	94	51	39
⩾1.5	93	54	41
⩾2.5	88	66	47
⩾3.0	65	84	58
⩾3.5	54	91	66
⩾4.0	42	93	67

Per potential total score, statistic diagnostic characteristics (sensitivity, specificity and positive predictive value) are given as percentages. For example, for a patient with anti-vimentin, anti-heparin sulphate and anti-histone H2B net fluorescent intensity values of 30, 50 and 150, respectively, a total score of 4.0 is attained (see Table 3). Thus, the chance that this person has NPSLE is 67% (positive predictive value corresponding to score 4.0).

IgM autoAb binding is often more variable, we focused on IgG autoAb reactivity because of the generally more robust and stable diagnostic detection rate. Therefore, IgG counterparts of IgM Abs were included in further analyses only if a significant correlation between these two parameters was present (supplementary Table S4, available at *Rheumatology* Online), leaving 12 antigens that were assessed further for their usefulness as a diagnostic marker for NPSLE (Table 2). Their validity as a diagnostic marker for discriminating NPSLE from non-NPSLE varied widely. In a receiver operating characteristic curve analysis, a low level of anti-vimentin IgG autoAbs (cut-off <40.5 NFI) demonstrated the best predictive value for the diagnosis NPSLE (87.0% sensitivity; 66.0% specificity; $P < 0.0001$).

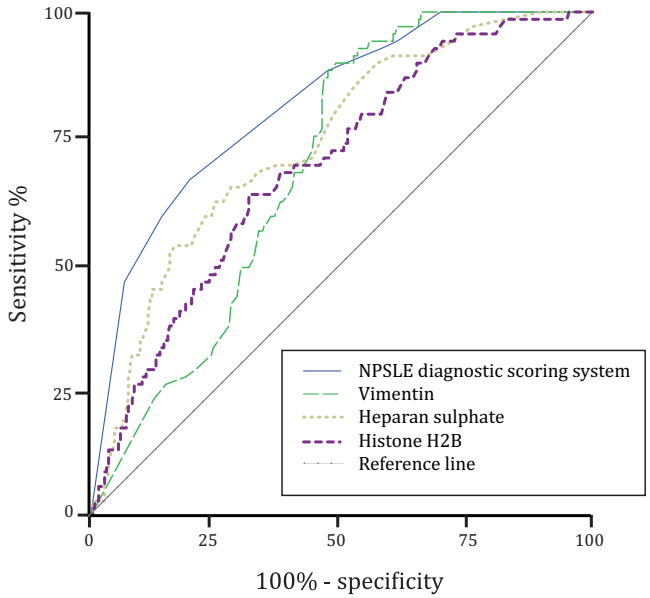
NPSLE diagnostic scoring system

A scoring system was established, based on cut-off NFI values of three IgG autoAbs (heparan sulphate, vimentin and histone H2B) as presented in Table 3. In this model, a higher score corresponds to an increased risk of having NPSLE (Table 4). The model provided good predictive value for the diagnosis of NPSLE [area under the curve 0.845 (99.97% CI: 0.756, 0.933; $P < 0.0001$; Fig. 2]. To exemplify, a patient with IgG anti-vimentin, anti-heparin sulphate and anti-histone H2B NFI values of 30, 50 and 150, respectively, attains a total score of 4.0. Although the sensitivity may still be limited, the proportion of patients with this score who had NPSLE is 67% (Table 4).

Discussion

For decades, researchers have tried to identify specific autoAbs as diagnostic markers for NPSLE patients [10]. It would be very useful to have predictive biomarkers to identify the risk for NP manifestations in SLE patients

Fig. 2 Receiver operating characteristic curves of the NPSLE diagnostic scoring system



Curves of the NPSLE diagnostic scoring system (continuous line) and the three autoantibodies that are included in the NPSLE diagnostic scoring system are plotted. The area under the curve is highest for the NPSLE diagnostic scoring system [0.851 (99.97% CI: 0.765, 0.937); $P < 0.0001$].

during the disease and to distinguish its two major phenotypes (inflammatory or ischaemic NPSLE).

In the present study, for the first time, we use multiplex autoAb microarrays to assess the association between autoAbs and the occurrence of NPSLE. IgG or IgM autoAbs that were significantly higher in NPSLE in comparison to non-NPSLE were directed against heparin, extracellular matrix proteins (amyloid and collagen II), a cellular membrane component (heparan sulphate), mitochondrial (cardiolipin and M2) and nuclear antigens (centromere protein A, Mi-2 and histones H2A and H2B; Fig. 1A–D). In contrast, autoAbs that were lower in NPSLE compared with non-NPSLE patients are phospholipid protein GP2, the cytoskeletal component vimentin, the coagulation factor prothrombin and nuclear antigens gP210 and Nup62 (Fig. 1A–D); although they were still significantly increased compared with healthy controls. Overall, NFI values of the IgG and IgM autoAbs differed considerably.

A valid NPSLE severity score based on clinical symptoms, imaging and laboratory results could guide treatment intensity and help in monitoring disease activity in clinical studies. Our study shows that a combination of autoAbs with distinctive NFI values (IgG autoAbs against heparan sulphate, vimentin and histone H2B; Fig. 2) may help to differentiate NPSLE from non-NPSLE. Once validated in prospective studies, our model might be clinically relevant in assessing the risk for NPSLE and in rational therapeutic decisions in case of SLE-related NP events. The NMDA receptor autoantibody, an interesting autoAb for NPSLE [15], was not included in our antigen panel. However, all SLE and NPSLE patients from the Academic Medical Centre, Amsterdam, The Netherlands, a tertiary national referral centre for NPSLE in the Netherlands, had been tested for the anti-NMDA receptor in serum, and results were negative (data not shown), although we cannot exclude the presence of any intrathecal autoantibodies [16].

Apart from its diagnostic use, it is possible that binding of these autoAbs to their cognate antigens is involved in NPSLE pathogenesis. Heparan sulphate is a complex polysaccharide of the cell membrane that binds numerous extracellular matrix proteins, and as such, aids in regulating a large variety of biological processes in, among others, endothelial cells and neuronal tissue [17–20]. Several researchers have stated that heparan sulphate is crucial in maintaining the stability of adherence junctions between brain endothelial cells [21]. Furthermore, dysfunction of this protein as a consequence of accumulation is associated with structural and functional blood–brain barrier impairment, neurodegeneration and neuroinflammation [22–25]. Inhibition of this protein by autoAbs could cause similar manifestations. Although heparan sulphate autoantibodies have been associated with lupus nephritis [11, 26], a diagnostically relevant role of anti-heparan in NPSLE is, to our knowledge, a new finding.

The second autoAb included in the NPSLE diagnostic scoring system, vimentin, is an intracellular intermediate filament protein expressed in mesenchymal cells [27]. It is known to be a protein with neuroprotective characteristics [28–30]. A higher vimentin concentration in astrocytes and endothelial cells is correlated with autoimmune

encephalomyelitis, neural trauma and neuronal oxidative stress [31–35]. The low anti-vimentin IgG we found in NPSLE was interesting, especially given the results of earlier studies, in which a positive association was found between anti-vimentin and primary or secondary APS and/or SLE [36, 37]. However, for the control and SLE group, we found similar associations (supplementary Tables S2 and S3, available at *Rheumatology* Online). To explain the lower levels of anti-vimentin IgG binding, although purely speculative, in NPSLE sera, post-translational modification of vimentin by processes such as citrullination or phosphorylation has been suggested [34, 35], resulting in decreased immunoreactivity in NPSLE patients as opposed to SLE patients. Another explanation for the low anti-vimentin level might be the occurrence of different splice variants of vimentin [38]. Anti-vimentin autoAbs could be directed against an epitope that is not present in the splice variant of vimentin as found in NPSLE. Moreover, clearance of vimentin from serum might result from enhanced exposition of vimentin in the brain, which may act as a sink (and disappearance of the autoAb from serum as a consequence). Finally, autoAbs against all histone subtypes and complexes have been associated with SLE before [39–41]. Moreover, IgG autoAbs against total histones and histone H2A have previously been associated with NPSLE [42, 43], and this was confirmed in our more extensive autoAb study (Fig. 1A–D).

Focusing on autoAb reactivity in inflammatory and ischaemic NPSLE, it can be stated that in addition to the more general presence of anti-histone H2A IgM autoAbs in both inflammatory and ischaemic NPSLE, anti-histone H2B IgM autoAbs seemed specifically to be associated with the inflammatory NPSLE subtype when compared with non-NPSLE patients (supplementary Fig. S3, available at *Rheumatology* Online). Surprisingly, our study suggests an inflammatory influence instead of an ischaemic role of anti-cardiolipin autoAbs in NPSLE. Although anti-cardiolipin autoAbs have been previously associated with thromboembolic events in SLE patients [7, 8], some studies also emphasized their inhibitory effect on astrocyte proliferation and their binding to brain components [44, 45]. Looking at autoAbs that were significantly different in ischaemic NPSLE compared with non-NPSLE, IgM autoAbs against collagen II seemed more specific for the ischaemic phenotype. IgG autoAbs against collagen II were proposed for monitoring SLE activity and disease-associated vasculitis [46, 47], supporting the vascular nature of the pathology in ischaemic NPSLE. However, we need to put these results into perspective owing to the finding that direct comparison of autoAbs between ischaemic and inflammatory NPSLE yielded no significant differences. This could be attributable to the relatively small number of patients tested with each phenotype, while also disregarding any potential overlap between the two phenotypes.

Although our study provides the most extensive data on autoAbs associated with SLE and NPSLE, it also has some important limitations, such as its cross-sectional character. Prospective research to identify the predictive value of these autoAbs before NP manifestations are present in patients diagnosed with SLE is recommended. A second limitation of this study was that some demographic characteristics in the healthy control population were significantly different from characteristics in the SLE patients. However, this difference is not thought to influence the comparison of autoAb profiles in NPSLE and non-NPSLE sera. Another important drawback is the lack of internal validation of our diagnostic scoring system. Internal validation of our system using a 70% training and 30% validation group was considered, but unfortunately, the number of NPSLE patients was too small to perform a reliable internal validation [48]. A final limitation of the study is the use of one technique (high-throughput multiplexed assay) for analysing the autoAbs. This technique yields high standard deviations because of its multiplex character. Lastly, for some antigens, relatively low NFI values were observed. Therefore, further validation should be done using other techniques, such as ELISA. However, such tests are not yet available for several of the antigens tested in the multiplex approach.

To summarize, this study shows that patients with NPSLE share a profile of IgG and IgM autoAbs against 15 antigens that is distinct from those measured in non-NPSLE patients. A profile of IgG autoAbs against heparan sulphate, vimentin and histone H2B indicated the presence or absence of NPSLE, which may be clinically relevant because of the monitoring and therapeutic consequences in case of SLE-related NP events.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

References

1 Borchers AT, Naguwa SM, Shoenfeld Y, Gerschwin ME. The geoepidemiology of systemic lupus erythematosus. Autoimmun Rev 2010;9:277–87.

2 Hanly JG, Urowitz MB, Su L *et al*. Prospective analysis of neuropsychiatric events in an international disease inception cohort of patients with systemic lupus erythematosus. Ann Rheum Dis 2010;69:529–35.

3 Magro-Checa C, Zirkzee EJ, Huizinga TW, Steup-Beekman GM. Management of neuropsychiatric systemic lupus erythematosus: current approaches and future perspectives. Drugs 2016;76:459–83.

4 Petri M, Orbai AM, Alarcón GS *et al*. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012;64:2677–86.

5 Bertsias GK, Boumpas DT. Pathogenesis, diagnosis and management of neuropsychiatric SLE manifestations. Nat Rev Rheumatol 2010;6:358–67.

6 The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. Arthritis Rheum 1999;42:599–608.

7 Zirkzee EJ, Steup-Beekman GM, van der Mast RC *et al*. Prospective study of clinical phenotypes in neuropsychiatric systemic lupus erythematosus; multidisciplinary approach to diagnosis and therapy. J Rheumatol 2012;39:2118–26.

8 Zandman-Goddard G, Chapman J, Shoenfeld Y. Autoantibodies involved in neuropsychiatric SLE and antiphospholipid syndrome. Semin Arthritis Rheum 2007;36:297–315.

9 Kivity S, Agmon-Levin N, Zandman-Goddard G, Chapman J, Shoenfeld Y. Neuropsychiatric lupus: a mosaic of clinical presentations. BMC Med 2015;4:13–43.

10 Sciascia S, Bertolaccini ML, Roccatello D, Khamashta MA, Sanna G. Autoantibodies involved in neuropsychiatric manifestations associated with systemic lupus erythematosus: a systemic review. J Neurol 2014;261:1706–14.

11 Li QZ, Xie C, Wu T *et al*. Identification of autoantibody clusters that best predict lupus disease activity using glomerular proteome arrays. J Clin Invest 2005;115:3428–39.

12 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.

13 Li Q-Z, Zhou J, Wandstrat AE *et al*. Protein array autoantibody profiles for insights into systemic lupus erythematosus and incomplete lupus syndromes. Clin Exp Immunol 2007;147:60–70.

14 Wessels JAM, Kooij van der SM, Cessie S *et al*. A clinical pharmacogenetic model to predict the efficacy of methotrexate monotherapy in recent-onset rheumatoid arthritis. Arthritis Rheum 2007;6:1765–75.

15 Hirohata S, Arinuma Y, Yanagida T, Yoshio T. Blood-brain barrier damages and intrathecal synthesis of anti-N-methyl-D-aspartate receptor NR2 antibodies in diffuse psychiatric/neuropsychological syndromes in systemic lupus erythematosus. Arthritis Res Ther 2014;16:R77.

16 Lauvsnes MB, Beyer MK, Kvaløy JT *et al*. Association of hippocampal atrophy with cerebrospinal fluid antibodies against the NR2 subtype of the *N*-methyl-D-aspartate receptor in patients with systemic lupus erythematosus and patients with primary Sjögren's syndrome. Arthritis Rheumatol 2014;66:3387–94.

17 Mercier F, Arikawa-Hirasawa E. Heparan sulfate niche for cell proliferation in the adult brain. Neurosci Lett 2012;510:67–72.

18 Okolicsanyi RK, Griffiths LR, Haupt LM. Mesenchymal stem cells, neural lineage potential, heparan sulfate proteoglycans and the matrix. Dev Biol 2014;388:1–10.

19 Kerever A, Mercier F, Nonaka R *et al*. Perlecan is required for FGF-2 signaling in the neural stem cell niche. Stem Cell Res 2014;12:492–505.

20 Edwards TJ, Hammarlund M. Syndecan promotes axon regeneration by stabilizing growth cone migration. Cell Rep 2014;8:272–83.

21 Steiner E, Enzmann GU, Lyck R *et al*. The heparan sulfate proteoglycan agrin contributes to barrier properties of mouse brain endothelial cells by stabilizing adherens junctions. Cell Tissue Res 2014;358:465–79.

22 Garbuzova-Davis S, Louis MK, Haller EM *et al*. Blood-brain barrier impairment in an animal model of MPS III B. PLoS One 2011;6:e16601.

23 Garbuzova-Davis S, Mirtyl S, Sallot SA *et al*. Blood-brain barrier impairment in MPS III patients. BMC Neurol 2013;13:174.

24 Voyvodic PL, Min D, Liu R *et al*. Loss of syndecan-1 induces a pro-inflammatory phenotype in endothelial cells with a dysregulated response to atheroprotective flow. J Biol Chem 2014;289:9547–59.

25 Martins C, Hůlková H, Dridi L *et al*. Neuroinflammation, mitochondrial defects and neurodegeneration in mucopolysaccharidosis III type C mouse model. Brain 2015;138:336–55.

26 Ravirajan CT, Rowse L, MacGowan J, Isenberg DA. An analysis of clinical disease activity and nephritis-associated serum autoantibody profiles in patients with systemic lupus erythematosus: a cross-sectional study. Rheumatology 2001;40:1405–12.

27 Bruyère J, Roy E, Ausseil J *et al*. Heparan sulfate saccharides modify focal adhesions: implication in mucopolysaccharidosis neuropathophysiology. J Mol Biol 2015;427:775–91.

28 Mendez MG, Restle D, Janmey PA. Vimentin enhances cell elastic behavior and protects against compressive stress. Biophys J 2014;107:314–23.

29 Jeong HK, Jou I, Joe EH. Absence of delayed neuronal death in ATP-injected brain: possible roles of astrogliosis. Exp Neurobiol 2013;22:308–14.

30 Liu Z, Li Y, Cui Y *et al*. Beneficial effects of gfap/vimentin reactive astrocytes for axonal remodeling and motor behavioral recovery in mice after stroke. Glia 2014;62:2022–33.

31 Guo F, Maeda Y, Ma J *et al*. Macroglial plasticity and the origins of reactive astroglia in experimental autoimmune encephalomyelitis. J Neurosci 2011;31:11914–28.

32 Ekmark-Lewén S, Lewén A, Israelsson C *et al*. Vimentin and GFAP responses in astrocytes after contusion trauma to the murine brain. Restor Neurol Neurosci 2010;28:311–21.

33 Ning M, Sarracino DA, Kho AT *et al*. Proteomic temporal profile of human brain endothelium after oxidative stress. Stroke 2011;42:37–43.

34 Liu T, Guevara OE, Warburton RR *et al*. Regulation of vimentin intermediate filaments in endothelial cells by hypoxia. Am J Physiol Cell Physiol 2010;299:363–73.

35 Rudrabhatla P, Jaffe H, Pant HC. Direct evidence of phosphorylated neuronal intermediate filament proteins in neurofibrillary tangles (NFTs): phosphoproteomics of Alzheimer's NFTs. FASEB J 2011;25:3896–905.

36 Ortona E, Capozzi A, Colasanti T *et al*. Vimentin/cardiolipin complex as a new antigenic target of the antiphospholipid syndrome. Blood 2010;116:2960–7.

37 Ripoll VM, Lambrianides A, Pierangeli SS *et al*. Changes in regulation of human monocyte proteins in response to IgG from patients with antiphospholipid syndrome. Blood 2014;124:3808–16.

38 von Brandenstein M, Puetz K, Schlosser M *et al*. Vimentin 3, the new hope, differentiating RCC versus oncocytoma. Dis Markers 2015;2015:368534.

39 Cohen MG, Pollard KM, Webb J. Antibodies to histones in systemic lupus erythematosus: prevalence, specificity, and relationship to clinical and laboratory features. Ann Rheum Dis 1992;51:61–6.

40 Stummvoll GH, Fritsch RD, Meyer B *et al*. Characterisation of cellular and humoral autoimmune responses to histone H1 and core histones in human systemic lupus erythaematosus. Ann Rheum Dis 2009;68:110–6.

41 Wayaku T, Hasegawa M, Kaji K *et al*. Antigen specificity of antihistone antibodies in connective tissue disease patients with anti-U1RNP antibodies. Rheumatol Int 2007;28:113–9.

42 Iizuka N, Okamoto K, Matsushita R *et al*. Identification of autoantigens specific for systemic lupus erythematosus with central nervous system involvement. Lupus 2010;19:717–26.

43 Sun XY, Shi J, Han L, Su Y, Li ZG. Anti-histones antibodies in systemic lupus erythematosus: prevalence and frequency in neuropsychiatric lupus. J Clin Lab Anal 2008;22:271–7.

44 Sun KH, Liu WT, Tsai CY *et al*. Inhibition of astrocyte proliferation and binding to brain tissue of anticardiolipin antibodies purified from lupus serum. Ann Rheum Dis 1992;51:707–12.

45 Lai NS, Lan JL. Evaluation of cerebrospinal anticardiolipin antibodies in lupus patients with neuropsychiatric manifestations. Lupus 2000;9:353–7.

46 He C, Mao T, Feng Y *et al*. Anti-CII antibody as a novel indicator to assess disease activity in systemic lupus erythematosus. Lupus 2015;24:1370–6.

47 Sjoholm AG, Martensson U, Sturfelt G. Serial analysis of autoantibody responses to the collagen-like region of Clq, collagen type II, and double stranded DNA in patients with systemic lupus erythematosus. J Rheumatol 1997;24:871–8.

48 Ogundimu EO, Altman DG, Collins GS. Adequate sample size for developing prediction models is not simply related to events per variable. J Clin Epidemiol 2016;76:175–82.