On cerebral lupus: from pathogenesis to clinical outcomes

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COMPLEMENT LEVELS AND ANTI-C1Q AUTOANTIBODIES IN PATIENTS WITH NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

Objective: To analyse serum levels of anti-C1q, C1q circulating immune complexes (CIC), complement activation and complement components in systemic lupus erythematosus (SLE) patients during the first central nervous system neuropsychiatric (NP) event and to define the possible association between these results and clinical and laboratory characteristics.

Methods: A total of 280 patients suspected of having NP involvement due to SLE were recruited in the Leiden NP-SLE-clinic. All SLE patients were classified according to the ACR 1982 revised criteria for the classification of SLE. The clinical disease activity was measured by the SLE Disease Activity Index 2000 (SLEDAI-2K) and NP diagnoses were classified according to the 1999 ACR case definitions for NP-SLE. We measured in serum of all patients anti-C1q and C1q CIC levels, the activation capacity of complement (CH50 and AP50) and different complement components (C1q, C3, C4).

Results: In 92 patients the symptoms were attributed to SLE. NP-SLE patients consisted of 63 patients with focal NP-SLE and 34 patients with diffuse NP-SLE. Anti-C1q antibodies were significantly higher and CH50, AP50 and C3 were significantly lower in NP-SLE patients compared with SLE patients without NP-SLE. This association was specially marked for diffuse NP-SLE while no differences were found for focal NP-SLE. After using potential predictors, decreased C4 remained significantly associated with focal NP-SLE, but only when antiphospholipid antibodies (aPL) were included in the model. C3 and AP50 were independently associated with diffuse NP-SLE. When SLEDAI-2K was included in the model these two associations were lost. When individual NP-SLE syndromes were analyzed, psychosis and cognitive dysfunction showed significantly lower values of complement activation capacity and all complement components. No significant associations were seen for other individual NP-SLE syndromes.

Conclusion: The associations between diffuse NP-SLE and anti-C1q, C3/AP50 and focal NP-SLE and C4 may be explained by disease activity and the presence of aPL respectively. The role of complement activation and complement components in lupus psychosis and cognitive dysfunction merits further research.

The complement system plays an important role in systemic lupus erythematosus (SLE). (1) Decreased levels of complement components, complement activation and higher levels of antibodies against C1q (anti-C1q) are characteristic findings in active SLE. A correlation between renal involvement and circulating immune complexes (CIC), complement deposits and levels of anti-C1q has been found in SLE.(1-3) However, the pathogenic role of all these complement components in other organs, including the nervous system, is less clear.

Complement factors are known to contribute to the pathology of inflammatory central nervous system (CNS) and neurodegenerative diseases and they have been proposed as one of the multiple participants in the pathogenesis of neuropsychiatric systemic lupus erythematosus (NP-SLE).(4-6) Data from human studies are scarce and contradictory. Although the exact underlying mechanism remains unknown, complement may collaborate in blood-brain barrier (BBB) alteration, brain cell dysfunction or vasculopathy and accelerated atherosclerosis. (5,7,8) Some authors have found an association between NP-SLE and low serum levels of C3 and C4 complement components, while increased levels of these proteins and the soluble form of C5b-9 have been found in the cerebrospinal fluid (CSF) of SLE patients.(9-11) An enhance deposition of complement activation products on platelets has also been associated with the development of thrombosis in SLE, a process where antiphospholipid antibodies (aPL) have been reported to be collaborate notably.(12,13)

In murine models, both deletion of factor B, a key alternative pathway protein, and inhibition of the classical and alternative complement cascade with the complement inhibitor Crry, demonstrated to alleviate experimental CNS lupus.(14,15) In addition, selective inhibition of two complement receptors, C3aR and C5aR, reduced neuronal degeneration (apoptosis and gliosis) and alleviated CNS lupus respectively.(16,17) C5 has also been reported to play a role in the maintenance of the BBB in a lupus rodent model.(18) Moreover, mice deficient in C3 and C5 components have also been reported to be resistant to enhanced thrombosis and endothelial cell activation induced by aPL antibodies, ameliorating the effect and pointing out the important role of alternative pathway complement activation on aPL-antibody mediated thrombogenesis.(19,20)

Serum complement levels are an accessible and worldwide used biomarker of great value for monitoring SLE activity. Although several studies have pointed out the role of the complement system in different aspects of NP-SLE pathogenesis, serum complement components (C1q, C3 and C4), the ability to activate the complement system (CH50, AP50), anti-C1q and C1q CIC have never been assessed in a large and well defined NP-SLE cohort. The aim of the current study was to analyze serum complement levels and anti-C1q levels during the first neuropsychiatric (NP) event of patients included in the Leiden NP-SLE-cohort, and to define the possible association between these results and clinical (NP-SLE syndromes, disease

activity and damage) and laboratory characteristics.

PATIENTS AND METHODS

Patient selection and clinical evaluation

From September 2007 until September 2014, 280 consecutive patients suspected of having NP involvement due to SLE were referred to the Leiden NP-SLE-clinic (Leiden University Medical Center, The Netherlands) for evaluation. All the subjects were admitted for 1-day and underwent multidisciplinary examination including neuropsychological testing, as well as extensive laboratory and radiological examination. A multidisciplinary consensus meeting took place soon after the evaluation of every patient. For further description of the multidisciplinary evaluation, please see reference (21). All the patients were classified according to the American College of Rheumatology (ACR) 1982 revised criteria for the classification of SLE.(22,23) The clinical disease activity was measured by the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) patient.(24) For the better assessment of the effect of disease activity we decided to exclude the NP manifestations from the SLEDAI-2K. In the NP-SLE group we included all patients having at least one NP-SLE manifestation involving the CNS. NP diagnoses were classified according to the 1999 ACR case definitions for NP-SLE syndromes and classified into focal and diffuse NP-SLE according to these definitions.(21,25) All patients with antiphospholipid syndrome (APS) had a history of anticardiolipin IgG or IgM (aCL), anti-beta2 glycoprotein 1 IgG or IgM (anti-β2GP1) and/or positive lupus anticoagulant (LAC) tests documented on two or more occasions at least 3 months apart. Furthermore, all these patients met the Sapporo clinical criteria.(26) In addition, 200 healthy controls (HC), aged between 20 and 70 years, were included in this study. All participants in the study provided informed consent and the study was approved by the local medical ethics committee.

Laboratory assessment

Serum samples of all patients were collected from each subject at 08:00 AM after overnight fasting. The functional capability of the complement components to activate the complement system of the classical pathway (CH50) and the alternative pathway (AP50) and levels of complement components (C1q, C3 and C4) were measured the same day of the blood extraction in the routine clinical laboratory at the Leiden University Medical Center (LUMC), The Netherlands. CH50 and AP50 were measured using functional assays. Levels of C1q, C3 and C4 in serum were measured using laser nephelometry. Based on the normal limits for our laboratory, CH50 level < 74%, AP50 < 39%, C1q < 102 mg/l, C3 < 0.9 g/l and C4 < 95 mg/l were defined as low. Plasma was also prepared by centrifugation and aliquoted (500 µl) into polypropylene tubes before freezing and stored at - 80°C. Patient´s sera were kept frozen

until it was analyzed for the levels of anti-C1q and C1q CIC by enzyme-linked immunosorbent assay (ELISA). These laboratory determinations were performed at the Rheumatology Laboratory (LUMC, The Netherlands). Anti-C1q antibodies and C1q CIC in serum were measured by the QUANTA Lite ™ Anti-C1q ELISA and with the usage of the QUANTA Lite® C1q CIC ELISA (Inova Diagnostics, San Diego, CA, USA), following the protocol from the manufacturer. The reference intervals were defined as < 20 units/ml for anti-C1q and as < 4.4 µg Eq/ml for C1q CIC. These classifications were also used to classify the healthy subjects. Another set of blood samples was tested for aPL, anti-dsDNA, anti-Sm, anti-RNP, anti-SSA/Ro52 and anti-SSB/La antibodies in the routine clinical laboratory at the LUMC. IgG anti-dsDNA antibodies were detected using the Crithidia Luciliae indirect immune fluorescence technique (Immunoconcepts, Sacramento, USA). IgG antibodies against SS-A/Ro-52, SS-B/La, Sm, RNP and IgG and IgM anti-cardiolipine and anti-ß2-glycoproteine I antibodies were detected were determined using a Phadia® 250 EliA fluorescence enzyme immunoassay (FEIA) (Thermo Scientific, Freiburg, Germany). Lupus anticoagulans (LAC) was determined using STA-Rack en STA Evolution coagulation analysers (Stago, Parsippany, USA).

Statistical analysis

Patients with NP-SLE and SLE patients were compared with respect to demographic characteristics, clinical manifestations, autoantibody profile and complement components using $\chi 2$ test or with Fisher's exact test and Mann-Whitney U-test when appropriate. Differences in anti-C1q and C1q CIC between HC, SLE and NP-SLE or among NP-SLE subgroups were analyzed by the Kruskal-Wallis test with the Dunn multiple comparison test or the Mann-Whitney U-test when needed. Differences in CH50 and AP50 between groups were compared by using one-way ANOVA test. $\chi 2$ test and Fisher exact test were used to compare between NP-SLE subgroups (focal and diffuse NP-SLE) and individual NP-SLE syndromes and the complement components (C1q, C3 and C4). Odds ratios (OR) and 95% confidence intervals (CI) were also calculated. Five patients were included in both focal and diffuse NP-SLE groups. We preferred this situation over leaving these patients out of the study completely or leave them in only one of the two groups. Binary logistic regression was used to ascertain the effects of age, disease activity measured by SLEDAI-2K and different laboratory markers including antibodies and complement on the likelihood to have NP-SLE, focal NP-SLE or diffuse NP-SLE. Laboratory variables judged to have clinical relevance based on a priori knowledge and previous univariate analysis were retained in the final models. Variables of interest were evaluated in two models, one with complement components (C1q, C3, C4) and other with complement activation (CH50 and AP50), independently added to individual antibodies of interest (LAC, aCL, anti-dsDNA, anti-Sm and anti-C1q antibodies) and SLEDAI-2K. $p \leq 0.05$ was considered statistically significant. Statistical analysis was performed with commercially available software (IBM SPSS statistics, version 20.0 for

Table 1. Comparison clinical data SLE and NPSLE

	SLE n = 112	NPSLE		
		Total n = 92	Focal * n = 63	Diffuse * n = 34
Age, mean ± SD years	44.01 ± 13.78	**40 ± 13.68** [a]	43.23 ± 13.86	**33.21 ± 10.19** [b,d]
Sex, no. female/male	99/13	82/10	55/8	32/2
Age at diagnosis SLE, mean ± SD years	35.4 ± 14.93	32.45 ± 14.8	35.01 ± 15.98	**26.34 ± 10.05** [a,c]
SLE disease duration, mean ± SD years	8.61 ± 8.55	7. 83 ± 8.31	8.23 ± 8.7	7.57 ± 8.08
SLEDAI-2K	4 [0 – 19]	**6 [0 – 22]** [b]	**6 [0 – 22]** [b]	**9 [0 – 22]** [b,c]
ACR 1982 criteria for SLE †				
Malar Rash	54 (48.2)	34 (37)	21 (33.3)	15 (44.1)
Discoid rash	25 (22.3)	12 (13)	9 (14.3)	4 (11.8)
Photosensitivity	50 (44.6)	31 (33.7)	22 (34.9)	9 (26.5)
Oral ulcers	40 (35.7)	32 (34.8)	19 (30.2)	14 (41.2)
Arthritis	79 (70.5)	63 (68.5)	41 (65.1)	26 (76.5)
Serositis	30 (26.8)	30 (32.6)	22 (34.9)	11 (32.4)
Renal disorder	33 (29.5)	19 (20.7)	9 (14.3)	**12 (35.3)** [c]
Neurologic disorder	8 (7.1)	**25 (27.2)** [a]	14 (22.2)	12 (35.3)
Hematologic disorder	50 (44.6)	44 (47.8)	29 (46)	17 (50)
Immunologic disorder	78 (69.6)	71 (77.2)	49 (77.7)	26 (76.5)
Positive ANA	111 (99.1)	89 (96.7)	61 (96.8)	32 (94.1)
Autoantibodies and complement †				
aCL IgG	8 (7.1)	**27 (29.3)** [b]	**21 (33.3)** [b]	**7 (20.6)** [a]
aCL IgM	6 (5.4)	8 (8.7)	6 (9.5)	3 (8.8)
LAC	19 (17)	**43 (46.7)** [b]	**35 (55.5)** [b]	**12 (35.3)** [a,c]
Anti-β2GP1 IgG ††	6 (5.4)	**17 (18.5)** [a]	**13 (20.6)** [a]	5 (14.7)
Anti-β2GP1 IgM ††	2 (1.8)	5 (5.4)	5 (7.9)	1 (2.9)
Antinuclear antibody	75 (66)	**78 (84.8)** [a]	**53 (84.1)** [a]	**29 (85.3)** [a]
Anti-dsDNA	23 (20.5)	**33 (35.9)** [a]	**22 (34.9)** [a]	**14 (41.2)** [a]
ENA	66 (58.9)	48 (52.2)	32 (50.8)	20 (58.8)
Anti-SSA/Ro52	57 (50.9)	**30 (32.6)** [a]	**21 (33.3)** [a]	**11 (32.4)** [a]
Anti-SSB/La	19 (17)	8 (8.7)	5 (7.9)	4 (11.8)
Anti-RNP	12 (10.7)	18 (19.6)	11 (17.5)	8 (23.5)
Anti-Sm	4 (3.6)	**12 (13)** [a]	7 (11.1)	**6 (17.6)** [a]
C1q low	7 (6.3)	13 (14.1)	7 (11.1)	**8 (23.5)** [a]
C3 low	29 (25.9)	**42 (45.7)** [a]	24 (38.1)	**22 (64.7)** [b,c]
C4 low	27 (24.1)	30 (32.6)	14 (22.2)	**18 (52.9)** [a,c]
CH50	25 (22.3)	**37 (40.2)** [a]	19 (30.2)	**19 (55.9)** [b,c]
AP50	16 (14.3)	**27 (29.3)** [a]	14 (22.2)	**16 (47.1)** [b,c]
Anti-C1q high	34 (30.3)	**41 (44.6)** [a]	26 (41.3)	**17 (50)** [a]
C1q CIC high	43 (38.4)	40 (43.5)	27 (42.9)	15 (44.1)
Antiphospholipid syndrome				
APS diagnosis	4 (3.6)	**22 (23.9)** [b]	**26 (41.3)** [b]	**6 (17.6)** [a]
Arterial thrombosis ever	19 (17)	**48 (52.2)** [b]	**43 (68.3)** [b]	**7 (20.6)** [d]
Vascular thrombosis ever	6 (5.4)	**15 (16.3)** [a]	**13 (20.6)** [a]	3 (8.8)

aCL: anticardiolipin antibodies; ACR: American College of Rheumatology; ANA: antinuclear antibody; LAC: Lupus anticoagulant; NPSLE: neuropsychiatric SLE; SLE: systemic lupus erythematosus; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000.
* 5 patients were included in both groups
† Number and percentage per group
†† Only available in 150 (69/81) patients
a. P < 0.05 when compared with SLE
b. P < 0.001 when compared with SLE
c. P < 0.05 when compared with focal SLE
d. P < 0.001 when compared with focal SLE

Windows; SPSS, Chicago, IL, USA). Figures were performed using GraphPad Prism 6 for Mac OS X ver. 6.0b, Graph-Pad Software, Inc., San Diego, CA, USA.

RESULTS

Demographic data and clinical characteristics

A total of 280 patients were analyzed in our NP-SLE clinic and 204 fulfilled the ACR classification criteria for SLE.(22,23) In 112 SLE patients, the NP complaints were better explained by another cause. A NP-SLE syndrome involving the CNS was diagnosed in 92 (45.1%) of the SLE patients. Among the patients diagnosed with CNS NP-SLE, 144 different ACR NP syndromes were established. Thirty-four patients had at least one diffuse NP-SLE syndrome while 63 patients were diagnosed with at least one focal NP-SLE syndrome according to the ACR 1999 NP-SLE definitions. (25) Five patients were diagnosed with both focal and diffuse symptoms. Patient demographics relevant to the present study are shown in **Table 1.** A description of all CNS syndromes included in the study is shown in **Table 2.**

Table 2. Central nervous system NPSLE syndromes of patients included in the study (n = 92) [a]

Central nervous system NPSLE syndromes	n
Aseptic meningitis	1
Cerebrovascular disease	45
Demyelinating syndrome	1
Headache	11
Movement disorder	3
Transverse myelitis	6
Seizure disorder	12
Psychosis	11
Acute confusional state	3
Anxiety disorder	5
Cognitive disorder	28
Mood disorder	18
Diffuse vs. focal NPSLE syndromes	
Focal NPSLE *	63
Diffuse NPSLE **	34
Diffuse NPSLE without non-specific syndromes †	61
Focal NPSLE without non-specific syndromes ††	22

NPSLE: neuropsychiatric systemic lupus erythematosus; SLE: systemic lupus erythematosus.
a. Possible > 1 NPSLE syndrome per patient
* Focal neuropsychiatric-SLE: Aseptic meningitis, cerebrovascular disease, demyelinating syndrome, headache, movement disorder, transverse myelitis, seizure disorder.
** Diffuse neuropsychiatric-SLE: psychosis, acute confusional state, anxiety disorder, cognitive disorder, mood disorder.
† Non-specific diffuse NPSLE syndromes: mood disorder, anxiety and mild cognitive dysfunction.
†† Non-specific focal NPSLE syndromes: headache.

Relationship of anti-C1q antibodies and C1q CIC and SLE and NP-SLE

Using the recommended cut-off values by the manufacturer, the positivity rates of anti-C1q levels and C1q CIC in HC were 13.5% (27 of 200) and 19.5% (39 of 200), respectively. Prevalence of anti-C1q antibodies and C1q CIC in NP-SLE and SLE patients is shown in **Table 1**. Levels of anti-C1q antibodies were higher in patients with NP-SLE than in both SLE (median 16.9 versus 8.0; $P < 0.05$) and HC (16.9 versus 7.0; $P < 0,001$) (**Figure 1A**). The same trend was seen in the C1q CIC levels when SLE and NP-SLE were compared with HC (**Figure 1B**). As previously described by other authors, the prevalence of anti-C1q antibodies was significantly higher in SLE patients with renal involvement (OR=2.1, 95% CI 1.1–3.9, $P < 0.05$), positivity for anti-dsDNA (OR=5.1, 95% CI 2.6–9.7, $P < 0.001$), and anti-Sm antibodies (OR=5.9, 95% CI 1.8–19.2, $P < 0.001$).(3,27,28) We also found a higher prevalence of C1q CIC in SLE patients with renal involvement (OR=2.1, 95% CI 1.1–3.9, $P < 0.05$), positivity for anti-dsDNA (OR=3.8, 95% CI 2.1–7.4, $P < 0.001$), and anti-Sm antibodies (OR=4.9, 95% CI 1.5–15.9, $P < 0.05$). The titers of anti-C1q antibodies and C1q CIC were also correlated with the SLEDAI-2K scores ($P < 0.001$ and $P < 0.05$, respectively) (data not shown). Among NP-SLE subsets, anti-C1q antibodies were significantly elevated only in diffuse NP-SLE compared with the rest of SLE patients (20.8 versus 8.7; $P < 0.05$) or HC (20.8 versus 7; $P< 0.05$). No differences in levels were found for C1q CIC when SLE and NP-SLE patients where compared. Among the different NP-SLE syndromes, only headache showed a significantly higher prevalence of anti-C1q antibodies (OR=4, 95% CI 1.1–14.6, $P < 0.05$). No significant associations were found between individual NP-SLE syndromes and C1q CIC (**Figures 1F and 1G**).

CH50 and AP50 and NP-SLE

NP-SLE patients showed significantly lower CH50 values (78.1 versus 89.8; $P < 0.05$) (**Figure 1D**) and AP50 (55.8 versus 69.8; $P = 0.001$) than SLE patients (**Figure 1E**). When the different NP-SLE subgroups were analyzed, the levels of CH50 and AP50 were markedly lower in patients with diffuse NP-SLE (both $P < 0.001$) when compared with SLE patients. No differences were found for focal NP-SLE. We next examined the association between CH50 and AP50 with the different NP-SLE syndromes. As shown in **Figures 1K and 1L**, psychosis (OR=60, 95% CI 7.2–501, $P < 0.001$), headache (OR=5, 95% CI 1.4–18.3, $P < 0.05$), seizure (OR=6, 95% CI 1.7–20.9, $P < 0.05$) and cognitive dysfunction (OR=3.8, 95% CI 1.5–9.8, $P < 0.05$) had significantly higher prevalence of low AP50 when compared with SLE, while psychosis (OR=9.2, 95% CI 2.2–37.6, $P = 0.001$), cognitive dysfunction (OR=3.4, 95% CI 1.5–8.2), $P < 0.05$) and mood disorder (OR=3.5, 95% CI 1.2–9.7, $P < 0.05$) showed a significantly higher prevalence of low CH50. No significant associations were seen with other individual NP-SLE syndromes.

Circulating levels of C1q, C3 and C4 in relation to NP-SLE

A significantly higher prevalence of low C3 was shown in NP-SLE (OR=2.4, 95% CI 1.3–4.3, P < 0.05), and especially in diffuse NP-SLE patients (OR= 5.2, 95% CI 2.3–11.9, P < 0.001), when compared with SLE patients (**Figure 1I**). An association between NP-SLE patients and lower values of C4 and C1q was not found; however low levels of these components were more prevalent in diffuse NP-SLE (C4: OR= 3.5, 95% CI 1.5–7.8, P < 0.05; C1q: OR= 4.6, 95% CI 1.5–13.8, P < 0.05). No associations were found with focal NP-SLE. Patients with lupus psychosis showed higher prevalence of low C1q (OR=5, 95% CI 1.5–15.8, P < 0.05), C3 (OR=28.6, 95% CI 3.5–230.4, P < 0.001) and C4 (OR=3.8, 95% CI 1.1–13.3, P < 0.05) when compared with SLE. Patients with cognitive dysfunction showed also higher prevalence of low C1q (OR=5, 95% CI 1.5–15.8, P < 0.05), C3 (OR=4.4, 95% CI 1.8–10.5, P < 0.001) and C4 (OR=3.6, 95% CI 1.5–8.6, P < 0.05) when compared with SLE. An association between headache and higher prevalence of low C4 (OR=3.7, 95% CI 1.1–13.3, P < 0.05) was also found. No significant associations were seen with other individual NP-SLE syndromes (**Figures 1H – 1J**).

Complement activation and complement components as predictor of NP-SLE

When possible complement activating factors were included in the model, NP-SLE patients showed a positive significant association with aCL IgG (OR=3.1, 95% CI 1.2–7.8, $p < 0.05$), LAC (OR=3.2, 95% CI 1.6–6.5, $p = 0.001$) and AP50 (OR=0.985, 95% CI 0.975–0.996, $p < 0.05$) after controlling for age, anti-dsDNA, anti-Sm, anti-C1q and CP50. When complement components were included in the model aCL IgG and LAC remained significant. After using all the same potential predictors, only aPL IgG (OR=5.9, 95% CI 2.1–17.3, P < 0.001), LAC (OR=5.7, 95% CI 2.6–12.6, P < 0.001), and also C4 (OR=4.1, 95% CI 1.4–12.2, P < 0.05) remained significantly associated with focal NP-SLE. After adjusting for above listed covariates, diffuse NP-SLE was associated with a lower age (P < 0.05). When complement components were included in the model, C3 was significantly associated with diffuse NP-SLE (OR=3.5, 95% CI 1.4–8.5, P < 0.05). Furthermore, when complement activation instead of complement components were used in the model, AP50 was also significantly associated with diffuse NP-SLE (OR=0.972, 95% CI 0.957–0.988, P < 0.001). When SLEDAI-2K was included in the model we missed these two associations.

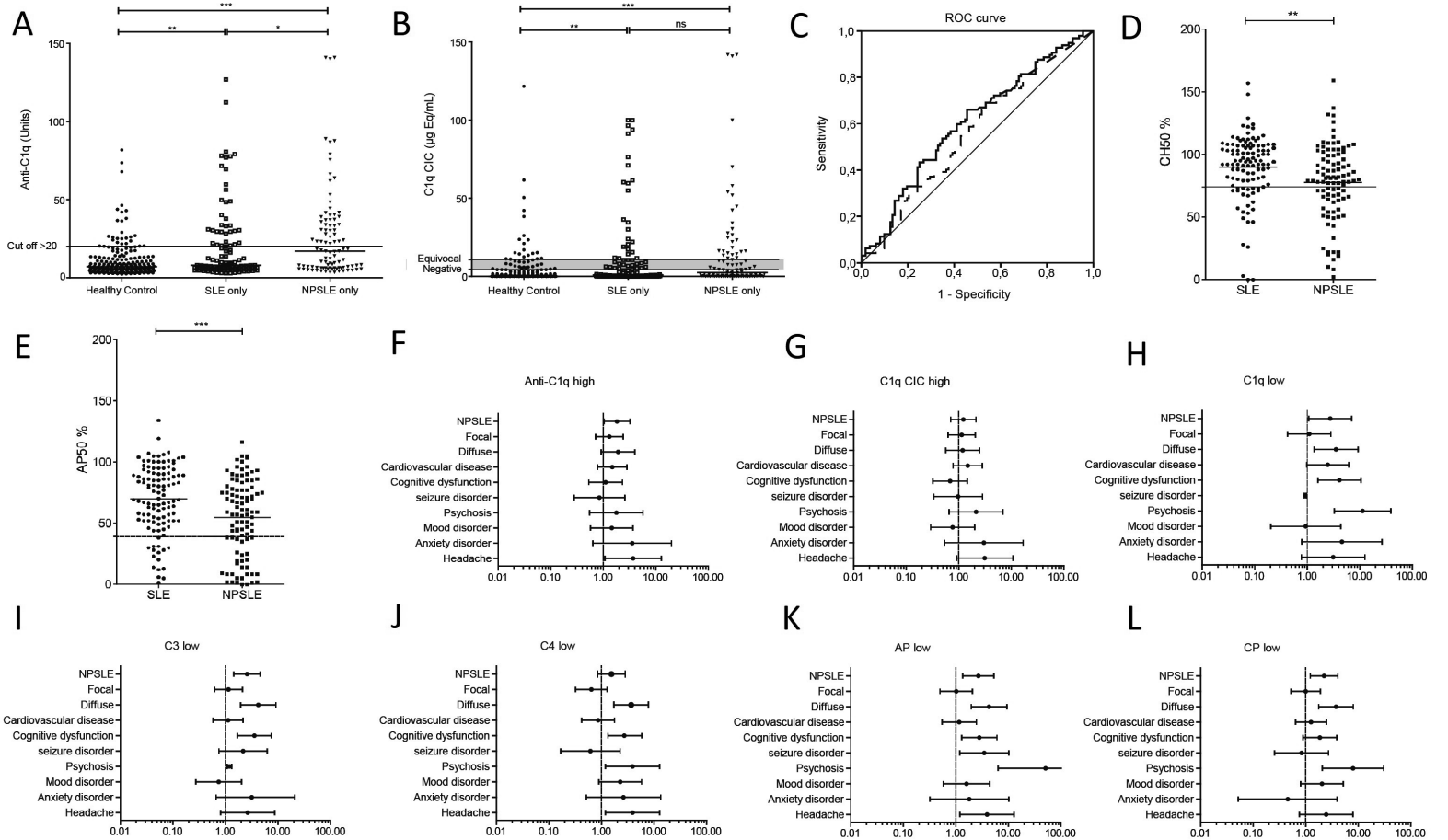
A
Anti-C1q (Units)
Cut off >20
Healthy Control
SLE only
NPSLE only
B
C1q CIC (µg Eq/mL)
Equivocal
Negative
ns
C
ROC curve
Sensitivity
1 - Specificity
D
CH50 %
SLE
NPSLE
E
AP50 %
F
Anti-C1q high
G
C1q CIC high
H
C1q low
I
C3 low
J
C4 low
K
AP low
L
CP low
NPSLE
Focal
Diffuse
Cardiovascular disease
Cognitive dysfunction
seizure disorder
Psychosis
Mood disorder
Anxiety disorder
Headache

Figure 1. Serum titers of (**A**) anti-C1q antibodies and (**B**) C1q CIC in 92 consecutive patients with NPSLE, 112 patients with SLE and 200 HC. The titers of anti-C1q antibodies were significantly higher in patients with NPSLE than in the rest of SLE patients and HC ($P < 0.05$ and $P < 0.001$, respectively). For anti-C1q antibodies and C1q CIC we indicate with a broken line the cut-off value recommended by the manufacturer. Horizontal lines indicate median. (**C**) Receiver-Operating-Characteristic (ROC) curves for the levels of anti-C1q and C1q-CIC in 112 patients with SLE and 92 patients with NPSLE. The mean (± SE) area under the curve for anti-C1q (continuous line) was 0.61 ± 0.04 and for C1q-CIC (dashed line) was 0.56 ± 0.04 for predicting NPSLE. Measurement of the activation state of the (**D**) classical pathway (CH50) and (**E**) alternative pathway (AP50) in 92 consecutive patients with NPSLE and 112 patients with SLE. For CH50 and AP50 we indicate with a broken line the cut-off value used in our laboratory. The levels of both CH50 and AP50 were significantly lower in NPSLE patients than in SLE ($P < 0.05$ for CH50 and $P < 0.001$ for AP50). Horizontal lines indicate mean. Odds ratios and 95% confidence interval analyzing the association of the more common NPSLE presentations in patients 204 SLE patients from the Leiden NPSLE-clinic. (**F**) Anti-C1q high as considered by manufacturer (> 20 U/ml), (**G**) C1q CIC high as considered by the manufacturer (> 4.4 µg Eq/ml), (**H**) low C1q measured using laser nephelometry (< 102 mg/l), (**I**) low C3 measured using laser nephelometry (< 0.9 g/l), (**J**) low C4 measured using laser nephelometry (< 95 mg/l), (**K**) low AP50 measured using functional assays (< 39%), and (**L**) CH50 measured using functional assays (<74%). **1A and 1B** Kruskal-Wallis test with Dunn's multiple comparison test and Mann-Whitney's U test, *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. **1D and 1E** One-way analysis of variance test, *$P < 0.05$, ** $P < 0.01$. **1F – 1L** χ^2 test and Fisher exact tests. HC: healthy controls; NPSLE: neuropsychiatric systemic lupus erythematosus; SE: standard error; SLE: systemic lupus erythematosus.

DISCUSSION

The pathogenic processes that lead to damage or dysfunction in the nervous system due to SLE remains poorly understood. Important associations have been reported between several autoantibodies and nervous system involvement in SLE, such as aPL and cerebrovascular disease and anti-ribosomal P and lupus psychosis. However, no specific autoantibodies have been identified and serological biomarkers for NP-SLE are extremely needed. The role played for other elements beyond autoantibodies in the NP-SLE pathogenesis remains unclear.(29,30) This study analyzes for the first time the serum complement components (C1q, C3 and C4), complement activation (CH50 and AP50), anti-C1q and C1q CIC in a large and well defined cohort of NP-SLE with CNS involvement. The results in the present study have disclosed that none of the complement elements studied is useful to differentiate between NP-SLE and SLE, but that some of them may be associated with a certain subset of NP-SLE patients.

We found an association between a low C4 and focal NP-SLE. Complement activation is known as an important mechanism of tissue injury in cerebral ischemia. Platelets bearing the complement activation product C4d are a known link between cerebrovascular inflammation and thrombosis. Moreover, they have been proposed as a specific biomarker for SLE diagnosis, and a relation with NP-SLE has also been suggested.(31,32) An increase in deposition of complement activation products, such as C4d, on platelets is associated with the presence of LAC, aCL and anti-β2GP1 antibodies and it has been proposed as an essential mechanism in aPL-mediated thrombosis in SLE.(12,13,31,32) Serum hypocomplementaemia is commonly

seen in patients with primary APS, reflecting complement activation and consumption.(33) It has been suggested that aPL may activate monocytes and macrophages via anaphylatoxins produced in complement activation.(33) An increase in complement activation products in serum of aPL positive patients has been related with the development of transient ischemic events and stroke.(34) In our cohort, the focal NP-SLE group was characterized by a higher prevalence of aPL and APS.(35) We have demonstrated that in this group the association with a low serum C4 was due to the association with the presence of LAC and aPL IgG. Serum C4 was not independently associated with focal NP-SLE or with cerebrovascular disease in SLE patients.

Diffuse NP-SLE patients were associated with a markedly low AP50 and low C3. Furthermore, we have shown for the first time that higher levels of anti-C1q antibodies are significantly associated with this NP-SLE subgroup when compared with SLE. Complement components C3 and C4 are recognized markers of global SLE activity and CH50 and AP50 are markedly reduced during SLE flares.(1,36) As reported in previous reports, we also observed an association between anti-C1q antibodies and known markers of global SLE activity such as SLEDAI-2K, anti-dsDNA antibodies, C1q CIC, C3, C4 and CH50.(27,37,38) Furthermore, similarly to other authors, we also confirmed a relation between anti-C1q levels and lupus nephritis (39,40) and younger age (28). Whereas in murine studies the association between anti-C1q autoantibodies and lupus nephritis has been well established (41,42), no such data is available to support the role of anti-C1q in other organ SLE manifestations. Diffuse NP-SLE manifestations have been linked to higher global SLE activity.(7) In our cohort we corroborate this association. We also miss the association between AP50 and low C3 and diffuse NP-SLE when SLEDAI-2K is included in the model. Since there is no gold-standard for NP-SLE, we cannot exclude the possibility that the multidisciplinary team that attributed the NP complaints to SLE was influenced by hypocomplementaemia when taking into account disease activity, which may explain our results. However, in clinical practice only hypocomplementaemia and not the evaluation of individual complement components have been taken into account. The fact that only AP50 and C3 and not CP and C4 were related with diffuse NP-SLE is intriguing and may be not biased by concomitant disease activity, leading us to make further interpretations. In murine models of lupus cerebritis, targeted and selective inhibition of the alternative complement pathway has been shown to be effective. (14-18) We could hypothesize that the complement alternative pathway may play a role in the pathogenesis of patients with diffuse NP-SLE.

Among the NP-SLE syndromes, patients with lupus psychosis had markedly higher complement activation and a higher prevalence of low serum C1q, C3 and C4. This association was especially marked for AP50 and C3. Lower serum C3 levels have been seen in corticosteroid-induced psychosis (43) and corticosteroid-induced psychiatric diseases

(44) in SLE patients. In the last case, C1q and C4 were also seen to be lower, however only serum C3 level was an independent risk factor for new-onset of psychiatric disorder after corticosteroid therapy.(45) Interestingly, complement activation was increasingly linked to schizophrenia development and psychopathology.(46) Some authors have reported lower levels of serum C3 in schizophrenia patients when compared with HC (47) whereas others have observed higher levels of C3 in these patients.(48) Also at the molecular level, the gene encoding C3, has been reported to be a genetic schizophrenia susceptibility region (49), whereas others could not confirm this.(50) In SLE patients data is limited. Pego-Reigosa et al. reported low C3 levels in 4/10 patients with lupus psychosis and no other complement alterations were found.(51) Watanabe et al. reported lower serum C3 levels in NP-SLE patients; however patients with lupus psychosis had higher serum C3 levels than other NP-SLE patients.(52) Further research on the link between alternative pathway and psychosis in patients with and without SLE, taking into account other factors such as corticosteroid treatment, is warranted.

Complement components C1q and C3 have emerged in the last years as key mediators of synaptic elimination and connectivity during development, normal ageing and neurodegeneration.(53-55) Complement has been localized at synapses and mediates pruning of synapses through a C3-dependent microglial phagocytosis process.(56) Cognitive decline, mediated through synapse elimination, has become a recognized feature in several neurodegenerative diseases.(57) For example, recent data in multiple sclerosis, an immune mediated inflammatory disease characterized for demyelination and leading memory impairment in up to 65% of patients, support that in the hippocampus of these patients there were clear signs of activation of complement components C1q-C3.(58) This disease shares some similarities with NP-SLE patients.(59) In our cohort, we found significantly lower levels of complement components, including C1q and C3, in patients with cognitive dysfunction due to SLE. The functional relationship between activation of complement components in brain pathology of NP-SLE patients should be investigated.

Although measuring complement activation by evaluating consumption of serum C3 and C4 are regularly used to track disease activity in SLE, the interpretation of these levels is challenging. They are acute phase reactants that may not decrease until late in a SLE flare. (60) Alterations in several components of the complement system in human CSF in NP-SLE patients have been scarcely studied. Higher levels of C3 and C4 have been reported in CSF when compared with controls. It has been proposed that this may reflect an intrathecal compensatory production (9). Intrathecal activation of terminal complement by measuring SC5b-9 in NP-SLE patients has also been seen. (11) Recent studies have demonstrated that several complement components are synthesized in the CNS (61) and also in human neuronal cells *in vitro*.(62) Autoantibodies in SLE are supposed to form immune complex

with complement (63) and induce neuroinflammation, but how this process occurs is far from clear.

Our study has notable limitations. Complement split products, which may reflect more accurately complement activation, were not evaluated. Furthermore, since lumbar puncture is not routinely performed in all the patients included in the NP-SLE-cohort, we lack the results of complement components in CSF. Determination of complement split products and parallel analysis of CSF must be included in future studies. Another limitation of our study is the retrospective design. On the other hand, all NP-SLE patients were unselected, consecutive patients, diagnosed in the same institution and in the same standardized multidisciplinary procedure. Our future work is aimed at prospectively finding associations between complement activation and components. Due to referral nuances, immunosuppressive therapy, including in some cases methylprednisolone, was already started in a few patients with diffuse NP-SLE patients. The effect of the therapy, mainly methylprednisolone, on complement component levels was not investigated. The small number of NP-SLE patients per syndrome may affect the power in this study and must be mentioned as a limitation. Definite conclusions concerning the relationship between complement components and NP-SLE syndromes cannot be drawn.

To our knowledge, this study is the first to investigate associations between complement elements measured in serum and clinical and serologic parameters in a large NP-SLE cohort. No association was found between anti-C1q or C1q CIC when all the NP-SLE patients where compared with SLE. We found an association between diffuse NP-SLE and anti-C1q, decreased C3 and AP50 and focal NP-SLE and decreased C4. These associations found between certain NP-SLE subgroups and several complement elements may be explained due to other factors such as aPL in the case of focal NP-SLE and global disease activity in the case diffuse NP-SLE. The roles of several complement aspects, especially alternative pathway activation and C3, in lupus psychosis and cognitive dysfunction merits further research.

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