The role of C1q in (auto) immunity

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Chapter 5

Complement levels and anti-C1q autoantibodies in patients with neuropsychiatric systemic lupus erythematosus

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Abstract

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 event and to define the possible association between these results and clinical and laboratory characteristics.

A total of 280 patients suspected of having NP involvement due to SLE were recruited in the Leiden NPSLE-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 NPSLE. 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).

In 92 patients the symptoms were attributed to SLE. NPSLE patients consisted of 63 patients with focal NPSLE and 34 patients with diffuse NPSLE. Anti-C1q antibodies were significantly higher and CH50, AP50 and C3 were significantly lower in NPSLE patients compared with SLE patients without NPSLE. This association was specially marked for diffuse NPSLE while no differences were found for focal NPSLE. After using potential predictors, decreased C4 remained significantly associated with focal NPSLE, but only when antiphospholipid antibodies (aPL) were included in the model. C3 and AP50 were independently associated with diffuse NPSLE. When SLEDAI-2K was included in the model these two associations were lost. When individual NPSLE 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 NPSLE syndromes.

The associations between diffuse NPSLE and anti-C1q, C3/AP50 and focal NPSLE 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.

Introduction

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 (NPSLE). [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 NPSLE 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 NPSLE 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 NPSLE 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 NPSLE-cohort, and to define the possible

association between these results and clinical (NPSLE 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 NPSLE-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 NPSLE group we included all patients having at least one NPSLE manifestation involving the CNS. NP diagnoses were classified according to the 1999 ACR case definitions for NPSLE syndromes and classified into focal and diffuse NPSLE 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 NPSLE 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 NPSLE or among NPSLE 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 NPSLE subgroups (focal and diffuse NPSLE) and individual NPSLE 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 NPSLE 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 NPSLE, focal NPSLE or diffuse NPSLE. 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 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 NPSLE 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 NPSLE syndrome involving the CNS was diagnosed in 92 (45.1%) of the SLE patients. Among the patients diagnosed with CNS NPSLE, 144 different ACR NP syndromes were established. Thirty-four patients had at least one diffuse NPSLE syndrome while 63 patients were diagnosed with at least one focal NPSLE syndrome according to the ACR 1999 NPSLE 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.

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

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 NPSLE and SLE patients is shown in Table 1. Levels of anti-C1q antibodies were higher in patients with NPSLE 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 NPSLE 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

	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

Table 1 Comparison clinical data SLE and NPSLE

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 NPSLE subsets, anti-C1q antibodies were significantly elevated only in diffuse NPSLE 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 NPSLE patients where compared. Among the different NPSLE 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 NPSLE syndromes and C1q CIC.

Central nervous system NPSLE syndromes	
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.

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

CH50 and AP50 and NPSLE

NPSLE patients showed significantly lower CH50 values (78.1 versus 89.8; P < 0.05) (Figure 2A) and AP50 (55.8 versus 69.8; P = 0.001) than SLE patients (Figure 2B). When the different NPSLE subgroups were analyzed, the levels of CH50 and AP50 were markedly lower in patients with diffuse NPSLE (both P < 0.001) when compared with SLE patients. No differences were found for focal NPSLE. We next examined the association between CH50 and AP50 with the different NPSLE syndromes. As shown in Figure 3, 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 NPSLE syndromes.

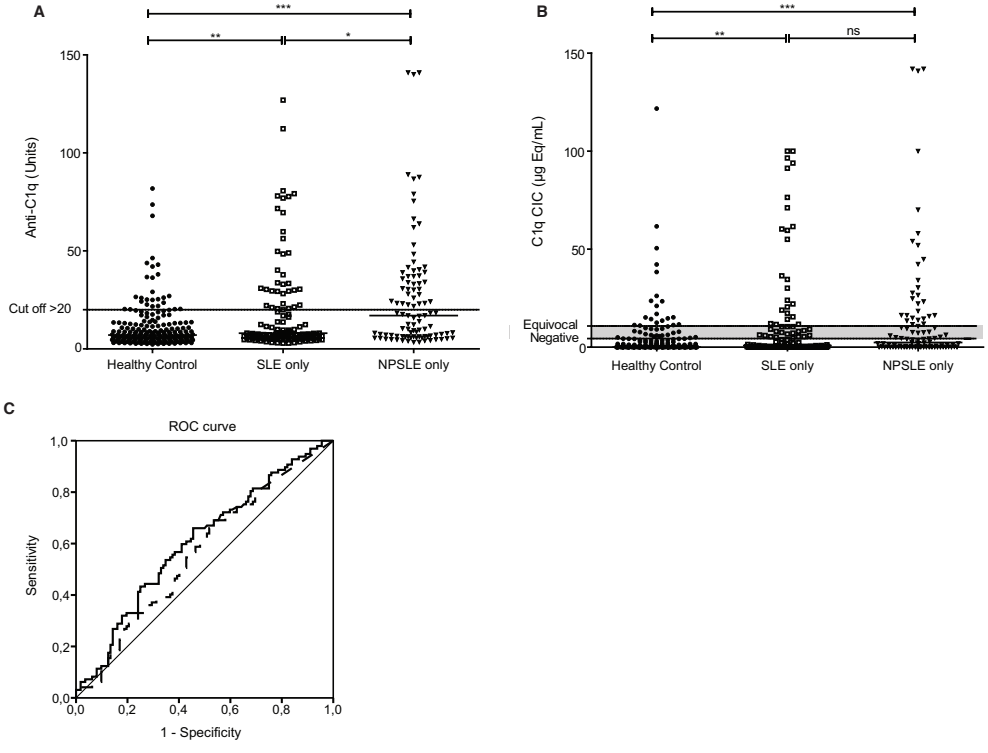

Figure 1. Serum titers of **A** anti-C1q antibodies and **B** C1q CIC in 92 consecutive patients with NPSLE, 112 patients with SLE and 200 HC. The titers of anti-C1q antibodies were significantly higher in patients with NPSLE than in the rest of SLE patients and HC (P < 0.05 and P < 0.001, respectively). 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.

HC: healthy controls; NPSLE: neuropsychiatric systemic lupus erythematosus; SE: standard error; SLE: systemic lupus erythematosus.

Kruskal-Wallis test with Dunn's multiple comparison test and Mann-Whitney's U test, *P < 0.05, ** P < 0.01, *** P < 0.001

Circulating levels of C1q, C3 and C4 in relation to NPSLE

A significantly higher prevalence of low C3 was shown in NPSLE (OR=2.4, 95% CI 1.3–4.3, P < 0.05), and especially in diffuse NPSLE patients (OR= 5.2, 95% CI 2.3–11.9, P < 0.001), when compared with SLE patients (Figure 3). An association between NPSLE patients and lower values of C4 and C1q was not found; however low levels of these components were more prevalent in diffuse NPSLE (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 NPSLE. 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 NPSLE syndromes.

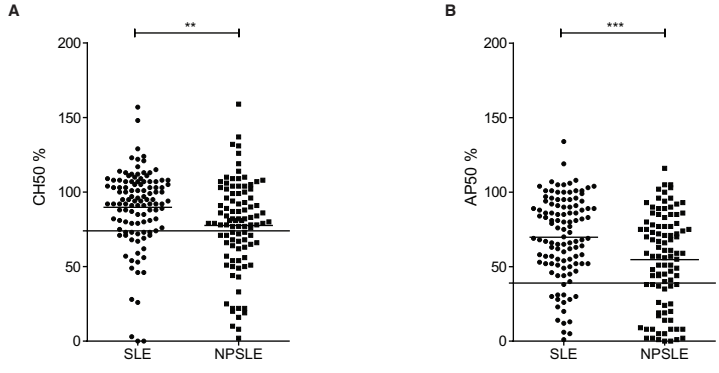

Figure 2. Measurement of the activation state of the **A** classical pathway (CH50) and **B** alternative pathway (AP50) in 92 consecutive patients with NPSLE and 112 patients with SLE. For CH50 and AP50 we indicate with a broken line the cut-off value used in our laboratory. The levels of both CH50 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.

NPSLE: neuropsychiatric systemic lupus erythematosus; SLE: systemic lupus erythematosus.

One-way analysis of variance test, *P < 0.05, ** P < 0.01

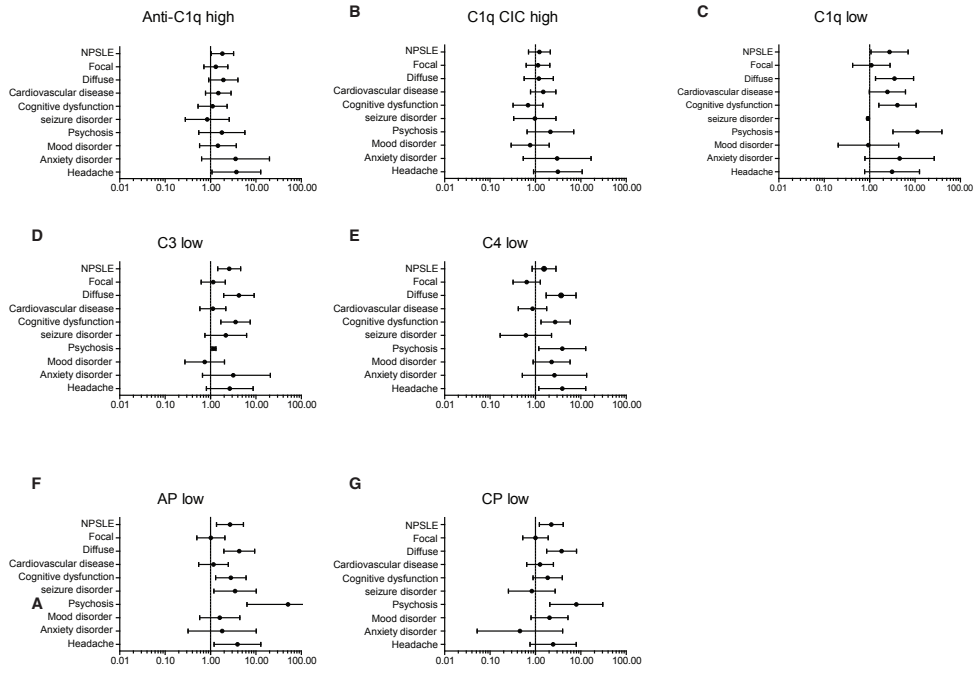

Figure 3. Odds ratios and 95% confidence interval analyzing the association of the more common NPSLE presentations in patients 204 SLE patients from the Leiden NPSLE-clinic. **A.** Anti-C1q high as considered by manufacturer (> 20 U/ml), **B.** C1q CIC high as considered by the manufacturer (> 4.4 µg Eq/ml), **C.** low C1q measured using laser nephelometry (< 102 mg/l), **D.** low C3 measured using laser nephelometry (< 0.9 g/l), **E.** low C4 measured using laser nephelometry (< 95 mg/l), **F.** low AP50 measured using functional assays (< 39%), and **G.** CH50 measured using functional assays (<74%).
NPSLE: neuropsychiatric systemic lupus erythematosus.
$\chi 2$ test and Fisher exact tests.

Complement activation and complement components as predictor of NPSLE

When possible complement activating factors were included in the model, NPSLE patients showed a positive significant association with aCL IgG (OR=3.126, 95% CI 1.2–7.8, $p < 0.05$), LAC (OR=3.233, 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=5.974, 95% CI 2.1–17.3, $P < 0.001$), LAC (OR=5.765, 95% CI 2.6–12.6, $P < 0.001$), and also C4 (OR=4.175, 95% CI 1.4–12.2, $P < 0.05$) remained significantly associated with focal NPSLE. After adjusting for above listed covariates, diffuse NPSLE was associated with a lower age ($P < 0.05$). When complement components were included in the model, C3 was significantly associated with diffuse NPSLE (OR=3.552, 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 NPSLE (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.

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 NPSLE are extremely needed. The role played for other elements beyond autoantibodies in the NPSLE 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 NPSLE with CNS involvement. The results in the present study have disclosed that none of the complement elements studied is useful to differentiate between NPSLE and SLE, but that some of them may be associated with a certain subset of NPSLE patients.

We found an association between a low C4 and focal NPSLE. 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 NPSLE 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 NPSLE 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 NPSLE or with cerebrovascular

disease in SLE patients.

Diffuse NPSLE 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 NPSLE 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 nepthritis has been wel established [41, 42], no such data is available to support the role of anti-C1q in other organ SLE manifestations. Diffuse NPSLE 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 NPSLE when SLEDAI-2K is included in the model. Since there is no gold-standard for NPSLE, 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 NPSLE 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 NPSLE.

Among the NPSLE 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 NPSLE patients; however patients with lupus psychosis had higher serum C3 levels than other NPSLE 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 NPSLE 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 NPSLE 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 NPSLE 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 NPSLE 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 NPSLE-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 NPSLE 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 NPSLE patients. The effect of the therapy, mainly methylprednisolone, on complement component levels was not investigated. The small number of NPSLE 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 NPSLE 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 NPSLE cohort. No association was found between anti-C1q or C1q CIC when all the NPSLE patients where compared with SLE. We found an association between diffuse NPSLE and anti-C1q, decreased C3 and AP50 and focal NPSLE and decreased C4. These associations found between certain NPSLE subgroups and several complement elements may be explained due to other factors such as aPL in the case of focal NPSLE and global disease activity in the case diffuse NPSLE. 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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