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Role of autoantibodies and blood–brain barrier leakage in cognitive impairment in systemic lupus erythematosus

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To cite: Hanly JG, Legge A, Kamintsky L, *et al*. Role of autoantibodies and blood–brain barrier leakage in cognitive impairment in systemic lupus erythematosus. *Lupus Science & Medicine* 2022;**9**:e000668. doi:10.1136/lupus-2022-000668

Received 21 January 2022
Accepted 20 May 2022

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ABSTRACT

Objective Cognitive impairment is common in patients with SLE but the cause is unknown. The current cross-sectional study examined the association between select SLE-related autoantibodies, other serological biomarkers and extensive blood–brain barrier (BBB) leakage in patients with SLE with and without cognitive impairment. In addition, we determined whether the relationship between SLE autoantibodies, other biomarkers and cognitive impairment differed depending on the presence or absence of concurrent extensive BBB leakage.

Methods Consecutive patients with SLE, recruited from a single academic medical centre, underwent formal neuropsychological testing for assessment of cognitive function. On the same day, BBB permeability was determined using dynamic contrast-enhanced MRI scanning. SLE autoantibodies and other serological biomarkers were measured. Regression modelling was used to determine the association between cognitive impairment, extensive BBB leakage and autoantibodies/biomarkers.

Results There were 102 patients with SLE; 90% were female and 88% were Caucasian, with a mean±SD age of 48.9±13.8 years. The mean±SD SLE disease duration was 14.8±11.0 years. Impairment in one or more cognitive tests was present in 47 of 101 (47%) patients and included deficits in information processing speed (9%), attention span (21%), new learning (8%), delayed recall (15%) and executive abilities (21%). Extensive BBB leakage was present in 20 of 79 (25%) patients and was associated with cognitive impairment (15 of 20 (75%) vs 24 of 59 (41%); p=0.01) and shorter disease duration (median (IQR): 7 (8–24 years) vs 15 (2–16 years); p=0.02). No serological parameters were associated with extensive BBB leakage and there was no statistically significant association between cognitive impairment and circulating autoantibodies even after adjusting for BBB leakage.

Conclusions Extensive BBB leakage alone was associated with cognitive impairment. These findings suggest that BBB leakage is an important contributor to cognitive impairment, regardless of circulating SLE-related autoantibodies.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Cognitive impairment is frequent in patients with SLE.
⇒ SLE-related autoantibodies have been proposed to cause cognitive impairment but to require the presence of increased blood–brain barrier (BBB) leakage.
⇒ We have recently reported an association between extensive BBB leakage detected by dynamic contrast-enhanced MRI and cognitive impairment in patients with SLE.

WHAT THIS STUDY ADDS

⇒ This cross-sectional study revealed that lupus-related autoantibodies were not associated with cognitive impairment even in the presence of extensive BBB leakage.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ The findings suggest that extensive BBB leakage is an important cause of cognitive impairment in patients with SLE, regardless of circulating lupus autoantibodies.
⇒ Future research on cognitive impairment in SLE should focus on the cause of the BBB abnormality and the downstream pathogenic mechanisms triggered by BBB dysfunction.

Neuropsychiatric (NP) events are frequent in patients with SLE and are associated with lower self-reported health-related quality of life and with increased mortality.[1] Cognitive impairment is one of the most frequent manifestations of neuropsychiatric SLE (NPSLE). In a meta-analysis of 2463 patients with SLE, the average prevalence of cognitive impairment was 38%. It was 1.8–2.8 times more frequent than in patients with rheumatoid arthritis or healthy controls, respectively.[2] In contrast to patients with neurodegenerative diseases, cognitive dysfunction in the majority of patients with SLE is not characterised by relentless progression. Longitudinal studies of patients with SLE over 2–10 years have demonstrated an evanescent trajectory with

stabilisation, resolution or recurrence of subtle but clinically significant impairments.[3–6]

The cause of cognitive impairment in SLE is unclear. Clinically overt NP events,[7] mood disorders,[8] vascular risk factors and medications[9] may contribute but do not appear to be the predominant cause of cognitive difficulties in most patients. Of the many SLE-related autoantibodies, only circulating antiphospholipid antibodies have shown an association with cognitive impairment in cross-sectional[10] and more consistently in longitudinal studies.[3 6 11] In theory, most SLE autoantibodies need to cross the blood–brain barrier (BBB) to gain access to the brain in order to exert a pathogenic effect. Historically, the assessment of BBB integrity has required access to paired serum and cerebrospinal fluid (CSF),[12] but a lumbar puncture is difficult to justify in patients with SLE who are not critically ill. In lieu of this invasive approach, we recently used dynamic contrast-enhanced MRI (DCE-MRI) to assess BBB integrity and reported an association between extensive BBB leakage and cognitive impairment in patients with SLE.[13] However, the potential link between cognitive impairment, BBB dysfunction and SLE-related autoantibodies requires further study.

The objectives of the current cross-sectional study were twofold: first, to determine if there was an association between select SLE-related autoantibodies or other serological biomarkers and extensive BBB leakage or cognitive impairment; and second to determine if the relationship of the same autoantibodies and biomarkers with cognitive impairment differed depending on the presence or absence of concurrent extensive BBB leakage.

METHODS

Patient characteristics

Patients fulfilling the revised American College of Rheumatology (ACR) criteria for SLE[14] were consecutively recruited from the Dalhousie Lupus Clinic, Division of Rheumatology, Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia. Patients were invited to participate in all components of the study, but DCE-MRI scanning was not conducted if the use of intravenous contrast was contraindicated or declined due to patient preference. No prescreening for cognitive impairment was performed. Participants provided written informed consent. Controls were healthy individuals between 35 and 70 years old with no NP history or chronic illness. This research was planned without patient involvement.

For patients with SLE, global SLE disease activity (Systemic Lupus Erythematosus Disease Activity Index-2000[15]) and cumulative organ damage (Systemic Lupus International Collaborating Clinics/ACR Damage Index[16]) were recorded (table 1). The Hospital Anxiety and Depression Scale questionnaire[17] was used to capture self-reported symptoms of depression and anxiety. Other variables included SLE-related medications such as corticosteroids, antimalarials, immunosuppressive drugs (methotrexate, azathioprine, cyclophosphamide, leflunomide, mycophenolate mofetil and intravenous gamma globulin) and biologic agents (rituximab or belimumab), use of psychoactive medications, lifestyle habits, and comorbidities (cigarette smoking, diabetes mellitus and hypertension). Laboratory variables included a complete blood count, serum creatinine, urinalysis, anti-double-straned (ds)DNA, antiphospholipid antibodies (anticardiolipin, anti-β2 glycoprotein I and lupus anticoagulant), and C3 and C4 levels. Blood collection, clinical and cognitive assessments, and MRI scanning were performed on the same day.

Cognitive function

Clinical neuropsychological tests completed by patients with SLE were based on ACR recommendations[18] focused on cognitive domains commonly affected in SLE.[19] Information processing speed and executive abilities were represented by the Symbol Digit Modalities Test[20] and the Design Fluency Test,[21] respectively. Components of the California Verbal Learning Test-II[22] provided indices of attention span (number of words recalled on trial 1), new learning (total words recalled over five list presentations) and delayed recall (number of words recalled after a 20 min delay). Raw scores were standardised based on published normative data and converted to Z-scores.[21] Those with Z-scores ≤−1.5 in one or more domains were deemed to have cognitive impairment.

MRI acquisition and analysis of BBB permeability

Quantitative assessment of BBB permeability was performed in 79 of 102 patients with SLE using DCE-MRI, as previously described.[13 23 24] Images were acquired using a 3T MRI scanner (Discovery MR750, GE Healthcare, Waukesha, Wisconsin), with a 32-channel MR Instruments head coil. The protocol included (1) a T1-weighted DCE sequence, acquired over a period of 20 min following an intravenous injection of a magnetic contrast agent (Gd-DOTA, Dotarem, Guerbet, France; 0.1 mmol/kg, 0.5 M, 1.5 mL/s; echo time (TE)/repetition time (TR): 2/4 ms; field-of-view (FOV): 240 mm; acquisition matrix: 192×192×34; voxel size: 1.25×1.25×6 mm; flip angle: 15°; Δt=4 s, 0–6 min postinjection; Δt=20 s); (2) a high-resolution anatomical scan (TE/TR: 2/6 ms; FOV: 224 mm; acquisition matrix: 224×224×168; voxel size: 1×1×1 mm; flip angle: 9°); and (3) a sequence of three scans acquired with variable flip angles (TE/TR: 2/10 ms; FOV: 240 mm; acquisition matrix: 192×192×34; voxel size: 1.25×1.25×6 mm; variable flip angles: 5°/10°/30°).

Determination of BBB permeability was estimated as previously described.[13 23 24] In brief, contrast extravasation due to cross-BBB leakage leads to increased T1-weighted signalling in the affected tissue, allowing the calculation of the contrast leakage rate for every brain voxel. To compensate for inter-subject variabilities (due to heart rate, blood flow or rate of contrast injection), each voxel's leakage rate was normalised to that of the superior sagittal sinus, resulting in a dimensionless leakage rate measure. With each voxel represented by the calculated leakage rate, three-dimensional maps of BBB

Table 1 Demographic and clinical features of patients with SLE and those with and without cognitive impairment

	SLE (n=102)	Cognitive impairment (n=47)	Normal cognition (n=54)	P value
Female, n (%)	92 (90)	43 (91)	49 (91)	
Age (years), mean±SD	48.9±13.8	50.2±13.3	47.5±14.4	0.33
Race/ethnicity, n (%)				
Caucasian	90 (88)	41 (87)	49 (91)	0.47
Other	11 (10.8)	6 (12.8)	5 (11.8)	
Years of education, mean±SD	15.6±3.4	14.8±2.9	16.3±3.7	**0.04**
Current smokers, n (%)	10 (9.8)	7 (15)	3 (6)	0.12
Ever smokers, n (%)	36 (35)	22 (47)	14 (26)	**0.03**
Number of pack years	5.1±10.2	7.1±11.0	3.4±9.1	**0.02**
SLE disease duration (years), mean±SD	14.8±11	16.9±12.4	12.6±9.0	0.11
Prior NP events (excluding cognitive impairment), n (%)	57 (56)	26 (55)	31 (57)	0.83
Prior NP events attributed to SLE (excluding cognitive impairment), n (%)	22 (22)	12 (26)	10 (19)	0.39
SLEDAI-2K score, mean±SD	2.62±2.94	3.19±3.62	2.07±2.09	0.17
SLEDAI-2K score without NP variables, mean±SD	2.62±2.94	3.19±3.62	2.07±2.09	0.17
SLICC/ACR Damage Index, mean±SD	1.03±1.43	1.21±1.52	0.87±1.36	0.26
SLICC/ACR Damage Index without NP variables, mean±SD	0.85±1.31	0.98±1.36	0.74±1.29	0.31
HADS depression score, mean±SD	4.7±3.86	5.3±4.18	4.22±3.55	0.26
HADS anxiety score, mean±SD	6.61±4.27	6.98±3.94	6.37±4.54	0.38
Medications, n (%)				
Corticosteroids	9 (8.8)	5 (10.6)	4 (7.4)	0.57
Antimalarials	77 (75.5)	31 (66.0)	46 (85.2)	0.023
Immunosuppressants	45 (44.1)	23 (48.9)	21 (38.9)	0.31
ASA/clopidogrel	12 (11.8)	6 (13.0)	6 (11.1)	0.79
Warfarin	8 (7.9)	6 (13.0)	2 (3.7)	0.86
Psychoactive drugs	41 (40.2)	21 (44.7)	20 (37.0)	0.44
Comorbidities, n (%)				
Hypertension	13 (12.7)	7 (15)	6 (11)	0.57
Diabetes	5 (4.9)	3 (6)	2 (4)	0.54

Bolded P values indicate statistically significant group differences between SLE patients with cognitive impairment and SLE patients with normal cognition.

ACR, American College of Rheumatology; ASA, Acetylsalicylic acid; HADS, Hospital Anxiety and Depression Scale; NP, neuropsychiatric; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index-2000; SLICC, Systemic Lupus International Collaborating Clinics.

leakage were constructed for each subject. Leakage rates were considered pathological when exceeding 0.02, the 95th percentile of all values in a cohort of control subjects.[23] The per cent of suprathreshold voxels was used as a measure reflecting whole-brain BBB leakage. Based on an outlier analysis of DCE-MRI scans of 65 patients with SLE (also included in the current study) and 9 healthy controls, extensive BBB leakage was defined as whole-brain values greater than 9.11% of brain volume.[13]

Autoantibodies, complement proteins and neurofilament light chain protein

The BioPlex 2200 system (Bio-Rad, Hercules, California)[25] was used to measure a panel of 'ANA screen' as per the manufacturer's directions. The panel included IgG autoantibodies to dsDNA, chromatin, ribosomal P, Ro (52 kD and 60 kD), La, Sm, ribonucleoprotein (RNP) (A and 68), Scl-70, Jo-1 and centromere B. Anti-dsDNA antibodies were quantified using serial dilutions of test samples if required and values ≥10 IU/mL were taken as positive. All other autoantibody specificities were reported as positive or negative using a cut-off level of antibody that corresponded to the 99th percentile of values obtained from normal controls provided by the manufacturer. A positive ANA was defined as the presence of one or more of the above autoantibody specificities.

IgG anticardiolipin antibodies (≥19 IgG phospholipid (GPL) units/mL) were measured on the BioPlex 2200 platform. IgG anti-β2 glycoprotein I antibodies were measured by ELISA using commercial kits (Inova Diagnostics, San Diego, California). Lupus anticoagulant measurement was based on the diluted Russell viper venom time (dRVVT) method using the dRVVT screen and dRVVT confirm reagents (HemosIL).

Binding assays were developed for measurement of IgG anti-N-methyl-D-aspartic acid receptor 2 (anti- NR2) antibodies to both linear (peptide target) and conformational (protein target) epitopes using two antigenic preparations: (1) NR2 peptide mix (NR2-1 [NH2]DWDYSVWLSN(X5)[COOH], NR2-2 [NH2] [Cys]DWEYSVWLSN[COOH] BSA, NR2-3 PEG12[NH2]DWDYSVWLSN[COOH], NR2-4 [NH2] [Cys] DWDYSVWLSN[COOH]-BSA); (2) NR2 protein mix (NR2A-protein 1 (partial length), from LSBio, Cat. LS-G25528 (31-555aa), and NR2A-protein 2 (partial length), from LSBio, Cat. LS-G22621 (501-550aa, 601-630aa, 701-750aa)). Additional assays measured IgG anti-glial fibrillar acidic protein (GFAP) antibodies (Recombinant Human GFAP Protein, Cat. ab114149; Abcam, Toronto, Ontario) and IgG anti-M-phase phosphoprotein 1 (HEK293T overexpressed KIF20B cell lysate)[26] antibodies. In all four assays, antigens were coupled to beads (MagPix Microspheres, Luminex, Austin, Texas) and incubated with diluted serum samples with gentle agitation for 30–60 min at room temperature. The beads were washed three times and 50 µL of diluted phycoerythrin-conjugated secondary antibody (Jackson ImmunoResearch, West Grove, Pennsylvania) were added and incubated with gentle agitation for another 30–60 min at room temperature in the dark. After the beads were washed three times, the plates were analysed using the MagPix System (Luminex). Data were expressed as the median fluorescence intensity and the cut-offs were established at 3 SD above the values observed in healthy adult control samples.

Plasma C3 and C4 levels were measured by immunoturbidimetry using Architect c16000 Chemistry Analyzer (Abbott Diagnostics, Mississauga, Ontario).

Neurofilament light chain levels were determined using the Two-Step Quanterix Simoa NF-Light Advantage Kit and analysed on the Quanterix SR-X Biomarker Detection System (Billerica, Massachusetts, USA). Briefly, all kit components and test samples were brought to room temperature prior to use. To generate a standard curve in determining sample neurofilament light concentrations, 100 µL of undiluted calibrators A-H (provided in the kit) were pipetted into provided 96-well plates. Test samples and controls were diluted 1:4 in a sample dilution buffer and 100 µL of each diluted test sample and controls were added to the plate. Calibrators, controls and test samples were incubated with 20 µL of detector reagent, 25 µL of neurofilament light chain-specific paramagnetic beads and 100 µL of streptavidin B-galactosidase as described by the manufacturer. Plates were processed using a BioTek 405TS Microplate Washer using a preset program specific for a Two-Step Quanterix assay. Following the processing procedure, the plate was analysed using the neurofilament light analysis protocol on a Quanterix SR-X Biomarker Detection System. Positive results were reported using age-adjusted normal reference ranges.

Statistical analysis

Descriptive statistics were reported as counts and percentages for categorical variables, mean and SD for normally distributed continuous variables, and median and IQR for non-normally distributed continuous variables. Patient characteristics were compared between those with and without extensive BBB leakage and between those with and without cognitive impairment. χ^2 or Fisher's exact test was used to compare categorical variables between groups and Wilcoxon rank-sum test was used for non-parametric continuous variables.

Separate multivariate logistic regression models were used to explore the association between primary outcome, cognitive impairment and each serological variable expressed as a dichotomous variable. Models were adjusted for occurrence of extensive BBB leakage. An interaction term was also included to determine if the relationship between the odds of cognitive impairment and each serological abnormality differed depending on whether there was extensive BBB leakage. Non-significant interaction terms at p>0.1 were omitted from the final models. Generalised additive models[16] were used to assess the association between cognitive impairment and each serological abnormality, expressed as a continuous variable. This method for non-parametric regression and smoothing approach relaxes the assumption of linearity and looks at the relationship between independent and dependent variables. Multivariate models were used to include extensive BBB leakage as a covariate. No interaction terms were included. P values <0.05 were considered statistically significant. SAS STAT 14.3 (V.9.4) was used for all analyses.

RESULTS

Demographic and clinical characteristics

There were 102 patients with SLE; 90% were female and 88% were Caucasian, with a mean±SD age of 48.9±13.8 years. The mean±SD SLE disease duration was 14.8±11.0 years and cumulative ACR classification criteria included malar rash (46 of 102, 45%), discoid rash (6 of 102, 6%), photosensitivity (51 of 102, 50%), oral/nasal ulcers (54 of 102, 53%), serositis (34 of 102, 33%), arthritis (78 of 102, 76%), renal disorder (30 of 102, 29%), neurological disorder (10 of 102, 10%), haematological disorder (91 of 102, 89%), immunological disorder (88 of 102, 86%) and ANA (102 of 102, 100%). Medication utilisation and autoantibodies reflected a general lupus population with low generalised disease activity and modest organ damage (table 1). Prior NP events from all causes occurred in 57 of 102 (56%) patients with SLE and NP events attributable to SLE were present in 22 of 102 (22%). The latter included transient ischaemic attacks

(n=5), stroke (n=3), seizure disorder (n=5), cranial neuropathy (n=4), acute confusional state (n=4), psychosis (n=2), major mood disorder (n=1), mononeuropathy (n=1), polyneuropathy (n=1) and aseptic meningitis (n=1).

Cognitive function

Neuropsychological testing was performed in 101 of 102 patients with SLE. One non-native English speaker was not tested. Impairment in one or more cognitive tests was present in 47 of 101 (47%) and included impairments in information processing speed (9%), attention span (21%), new learning (8%), delayed recall (15%) and executive abilities (21%). Patients with cognitive impairment had significantly fewer years of education and were more frequently smokers with more pack years. There were no significant differences in other demographic or clinical variables, including history of clinically overt NP events, current self-report anxiety or mood disorders, medication utilisation or frequency of medical comorbidities (table 1).

Extensive BBB leakage, clinical and demographic variables and autoantibodies

For the 79 patients who underwent DCE-MRI, the only variable listed in table 1 that was significantly different between the groups was higher use of acetylsalicylic acid (ASA)/clopidogrel in 12 of 79 (15%) patients who completed DCR-MRI compared with 0 of 23 (0%) patients who did not undergo DCE-MRI (p=0.047; without adjustment for multiple comparisons). Extensive BBB leakage was identified in 20 of 79 (25%) patients with SLE and was associated with a higher frequency of cognitive impairment (15 of 20 (75%) vs 24 of 59 (41%); p=0.01) and shorter disease duration (median (IQR): 7 (8–24 years) vs 15 (2–16 years); p=0.02). There was no association between autoantibodies or other serological variables with extensive BBB leakage (data not shown).

Autoantibodies and cognitive impairment

In the total SLE sample, there were no statistically significant associations between cognitive impairment and circulating autoantibodies, including antiphospholid (aPL), anti-ribosomal P and anti-NR2 (table 2). The only association that approached statistical significance was elevated anti-GFAP in 15 of 47 (32%) patients with cognitive impairment vs 9 of 54 (17%) patients with normal cognition (p=0.07). Hypocomplementaemia was not associated with concurrent cognitive impairment. Elevated neurofilament light chains were seen in a higher proportion of patients with cognitive impairment (13 of 47, 28%) compared with those with normal cognition (10 of 54, 19%), but this did not reach statistical significance (p=0.27). However, the absolute levels of neurofilament light chains were significantly higher in patients with cognitive impairment compared with those with normal cognition (mean±SD: 33.7±27.6 vs 21.5±12.2; p=0.03).

Table 2 Cognitive function and serological variables in patients with SLE

	SLE (n=102) n (%)	Cognitive impairment (n=47) n (%)	Normal cognition (n=54) n (%)	P value
Anti-dsDNA	43 (42)	20 (43)	22 (41)	0.85
Anti-chromatin	36 (35)	15 (32)	21 (39)	0.47
Anti-ribosomal P	7 (7)	2 (4)	4 (7)	0.50
Anti-Ro (60, 52)	37 (36)	18 (38)	19 (35)	0.75
Anti-La	30 (29)	15 (32)	15 (28)	0.99
Anti-Sm	17 (17)	7 (15)	10 (19)	0.63
Anti-RNP (A, 68)	29 (28)	12 (26)	17 (31)	0.51
Anticardiolipin	17 (17)	9 (20)	7 (13)	0.37
Anti-β2 glycoprotein I	14 (14)	8 (17)	5 (9)	0.25
LAC	23 (23)	13 (28)	9 (17)	0.18
Anticardiolipin or anti-β2 glycoprotein I or LAC	30 (30)	17 (36)	13 (24)	0.16
Anti-NR2 (peptide mix)	15 (15)	7 (15)	8 (15)	0.99
Anti-NR2 (protein mix)	12 (12)	4 (9)	8 (15)	0.31
Anti-GFAP	25 (25)	15 (32)	9 (17)	0.07
Anti-MPP1	34 (33)	14 (30)	19 (35)	0.56
Low C3	14 (14)	4 (9)	9 (17)	0.22
Low C4	29 (28)	15 (32)	13 (24)	0.38
Elevated neurofilament light chains	23 (23)	13 (28)	10 (19)	0.27

dsDNA, double-stranded DNA; GFAP, glial fibrillar acidic protein; LAC, lupus anticoagulant; MPP1, M-phase phosphoprotein 1; NR2, N-methyl-D-aspartic acid receptor 2; RNP, ribonucleoprotein.

Table 3 Logistic regression models for the association of serological variables with cognitive impairment in patients with SLE

Laboratory variable	OR (95% CI)	P value
Anti-dsDNA	0.92 (0.36 to 2.37)	0.87
Anti-chromatin	0.63 (0.24 to 1.68)	0.36
Anti-ribosomal P	N/A	N/A
Anti-Ro (60, 52)	0.878 (0.33 to 2.33)	0.79
Anti-La	1.10 (0.28 to 4.37)	0.89
Anti-Sm	0.74 (0.21 to 2.63)	0.64
Anti-RNP (A, 68)	0.61 (0.21 to 1.73)	0.35
Anticardiolipin	1.16 (0.33 to 4.10)	0.81
Anti-β2 glycoprotein I	1.66 (0.36 to 7.77)	0.52
LAC	2.11 (0.69 to 6.39)	0.19
Anticardiolipin or anti-β2 glycoprotein I or LAC	1.62 (0.59 to 4.45)	0.35
Anti-NR2 (peptide mix)	1.23 (0.32 to 4.67)	0.77
Anti-NR2 (protein mix)	0.55 (0.13 to 2.32)	0.42
Anti-GFAP	1.73 (0.57 to 5.29)	0.34
Anti-MPP1	0.45 (0.16 to 1.22)	0.12
Low C3	4.37 (1.34 to 14.23)	0.06
Low C4	1.13 (0.41 to 3.14)	0.81
Elevated neurofilament light chains	1.20 (0.41 to 3.51)	0.74

	NR2 protein mix MFI	NR2 peptide mix MFI	MPP1 MFI	GFAP MFI
Positive	≥1000	≥1000	≥1000	≥1000

dsDNA, double-stranded DNA; GFAP, glial fibrillar acidic protein; LAC, lupus anticoagulant; MFI, median fluorescence intensity; MPP1, M-phase phosphoprotein 1; N/A, not available due insufficient data for model; NR2, N-methyl-D-aspartic acid receptor 2; RNP, ribonucleoprotein.

Extensive BBB leakage, autoantibodies and cognitive impairment

There were no differences in the associations of autoantibodies or other serological variables with cognitive impairment based on the presence versus absence of extensive BBB leakage (p>0.1 for all interaction terms). As summarised in table 3, no significant association was observed between any of the autoantibodies or serological abnormalities and cognitive impairment after adjusting for extensive BBB leakage.

When autoantibodies and other serological abnormalities were modelled as continuous variables, the only statistically significant finding was that of higher neurofilament light chains in patients with SLE with cognitive impairment compared with those with normal cognition (median (IQR): 25.6 (13.6–42.4) vs 19.6 (12.6–27.2); p=0.03).

DISCUSSION

Proposed pathogenic mechanisms for nervous system disease that may manifest as cognitive impairment in persons with SLE include ischaemic injury and autoantibody-induced inflammation of the brain.[27] As suggested by work in animal models,[28 29] the latter requires a breach in the BBB in order for autoantibodies to access neuronal and other brain structures. Neuroimaging methods such as DCE-MRI provide direct and objective assessment of BBB permeability[13 23 30] and remove the need to sample CSF to obtain an indirect permeability index of the BBB. In the current study, we examined whether the co-occurrence of circulating lupus autoantibodies and extensive BBB permeability was associated with objectively determined cognitive impairment. Our findings indicate that none of the selected autoantibodies examined was associated with cognitive impairment, regardless of extensive BBB permeability. Rather, extensive BBB permeability alone was associated with cognitive impairment. This raises the possibility that while disruption of the BBB may lead to cognitive impairment in patients with SLE, it is not via mechanisms related to circulating lupus autoantibodies.

Autoantibodies are considered an integral component in the pathogenesis and end-organ disease of SLE. For example, serological–clinical associations include anti-dsDNA antibodies with lupus nephritis, anti-Ro60 antibody with subacute cutaneous lupus and neonatal lupus, and aPL antibodies with venous or arterial thrombosis. However, the role of autoantibodies in nervous system disease and cognitive impairment in patients with SLE is less clear.[31] The most consistent autoantibody association

reported with cognitive impairment is aPL antibodies, most likely by means of microvascular thrombosis.[3 6 10 11] In particular, longitudinal studies[3 6 11] have reported an association between persistent elevation of aPL antibodies and cognitive impairment. Other lupus autoantibodies such as anti-ribosomal P[28] and anti-NR2 antibodies[32] have been shown to bind to the surface of neuronal cells and induce hyperexcitability or apoptosis in vitro. However, the association between these and other peripheral blood autoantibodies and cognitive impairment in patients with SLE has been inconsistent.[33–36] One potential explanation is that unless the BBB is sufficiently permeabilised, circulating autoantibodies are unable to access neuronal tissue. However, the results of our study indicate that the presence of extensive BBB leakage does not strengthen the association of these autoantibodies with cognitive impairment. Rather the extensive BBB leakage per se is the strongest association with cognitive impairment, regardless of concurrent circulating autoantibodies .

The BBB is essential for brain health.[37] It consists of tightly connected endothelial cells surrounded by pericytes and astrocytes. A physical interface between the circulation and the brain, it also monitors and regulates the inflow and outflow of fluid, electrolytes and proteins to provide the optimal chemical environment required for normal neuronal function. Structural or functional breakdown in the BBB allows leakage of bloodborne products into the brain that can induce an inflammatory response that adversely affects glial function, extracellular matrix composition, neuronal connectivity and function. Extensive leakage of the BBB occurs in a variety of neurological disease states, including multiple sclerosis,[38] stroke,[39] traumatic brain injury[23] and dementia.[40]

In SLE there are a number of potential mechanisms that could cause injury or dysfunction of the BBB.[41] Lupus autoantibodies may bind to the surface of endothelial cells or other components of the BBB and induce a proinflammatory response with increased production of adhesion molecules, cytokines and complement activation. There is evidence from animal models and in vitro experiments to support such effects mediated by anti-ribosomal P[42] and anti-NR2 antibodies.[43] Although the results of the current study do not implicate these specific autoantibodies in BBB injury, it is possible that novel autoantibodies with other specificities play such a role. Non-SLE-specific mechanisms such as systemic infection, cigarette smoking and hypertension, all of which are more frequent in patients with SLE, can also increase BBB permeability. Extensive BBB leakage will provide unregulated access of proteins that may be harmful to the brain. In addition to autoantibodies, there are other proteins that can also have a harmful effect. One example is albumin,[44] which binds to astrocytes and induces the production of proinflammatory transforming growth factor beta, causing neuronal excitability and delayed neurodegeneration. Thrombin and activated protein C can also induce brain neuronal excitability.[45]

Most studies of biomarkers in NPSLE have been performed on CSF samples rather than peripheral blood.[46] Neurofilament light chain protein is part of the neuronal cytoskeleton and increased levels in the CSF reflect axonal damage and degeneration.[47 48] It is not disease-specific and has been used as a marker of neuronal injury in neurodegenerative disease,[49] multiple sclerosis,[47] cerebrovascular disease[48] and traumatic brain injury.[50] A recent study in patients with SLE and in patients with Sjögren's syndrome reported increased CSF levels of neurofilament light chain protein in association with cognitive impairment.[51] Increased CSF levels of neurofilament light chain protein have also been reported in patients with SLE with other clinical NP manifestations.[52] However, while CSF samples were not available in the current study, our finding of an association of cognitive impairment with peripheral blood neurofilament light chain protein levels provides further support on the importance of extensive BBB leakage and its utility as a biomarker of neuronal injury in patients with SLE, as seen in other neuroinflammatory and neurodegenerative disorders.[53] The trend towards statistical significance for the association between cognitive impairment and serum anti-GFAP antibodies warrants additional study in a larger SLE cohort in view of its known association with traumatic brain injury and with other neurological states in patients without SLE.[54]

There are strengths and limitations to our study. First, most patients had quiescent SLE without severe NP manifestations at the time of study. However, our patients reflected a typical ambulatory SLE population, although predominantly Caucasian, with the expected frequency and characteristics of cognitive impairment detected by formal neuropsychological assessment. Second, CSF samples were not available and may have provided a basis for more robust clinical–laboratory associations. Access to CSF would also have allowed the detection of intrathecal autoantibody production, although this occurs in a minority of patients with SLE.[12] DCE-MRI is an objective and relatively novel methodology for assessing BBB leakage in SLE and avoids the need to perform lumbar puncture to obtain CSF samples. A strength of the study was that neuroimaging, blood collection and cognitive assessments were performed on the same day and in the same sequence for each participant, thereby minimising the effect of a temporal disconnect between clinical, serological and neuroimaging variables of interest.

In summary, although causality cannot be inferred from our cross-sectional study, the findings suggest that perturbation of normal BBB function is related to cognitive impairment in a proportion of patients with SLE. Further work addressing the clinical and pathogenic bases for extensive BBB leakage is required as is further investigation aimed at understanding the mechanisms underlying cognitive impairment following BBB dysfunction in patients with SLE.

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Acknowledgements The authors thank Meifeng Zhang and Dr Paul Sciore (University of Calgary) for technical assistance with autoantibody and other biomarker testing.

Contributors All authors participated in the collection of research data, data analysis, or writing and critical review of the manuscript. JGH is the author acting as guarantor,

Funding This study was supported by the Canadian Institutes of Health Research (CIHR; MOP88526 and PJT148896, CIHR project grant 168878), Nova Scotia Health Research Foundation (NSHRF; MED-EST-2015-10067), Natural Sciences and Engineering Research Council of Canada (NSERC; RGPIN 04293-17 and RGPIN/05684-2016), Brain Canada (BC; PSG2015-3780), Mitacs (grant IT23320), Nova Scotia Research Fund, National MS Society, Research Nova Scotia, and consultation and distribution royalties from MAPI Research Trust. The funders of the study had no role in study design, patient recruitment, data collection, analysis and interpretation, or publication.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study involves human participants and was approved by the Nova Scotia Health Authority (NSHA) Research Ethics Board (study approval ID: 1021884). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. All data relevant to the study are included in the article.

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