

# Blood-based candidate biomarkers of the presence of neuropsychiatric systemic lupus erythematosus in children

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## ABSTRACT

**Objective:** To examine select brain-reactive proteins for their usefulness to serve as blood-based biomarkers in the screening for neurocognitive deficits in childhood-onset systemic lupus erythematosus (cSLE-NCD).

**Methods:** Patients with cSLE (n=40) were studied longitudinally (month 1; month 18): working memory, psychomotor speed and visuoconstructional ability were assessed using formal neurocognitive testing to determine the presence of cSLE-NCD. Patients also completed the computerised Paediatric Automated Neuropsychological Assessment Metrics. The following brain-reactive proteins were measured in the blood: neutrophil gelatinase associated lipocalin (NGAL), S100B, S100A8/9, antibodies to NR2 glutamate receptor (aNR2-AB), ribosomal-P (aP-AB), glycoprotein-1 (aGP1-AB), and lupus anticoagulant.

**Results:** cSLE-NCD was present in 6 of 40 patients at baseline and 4 of 27 patients with 18-month information. aP-AB positivity was more commonly present with cSLE-NCD than without (p=0.05). aP-ABs were negatively associated with performance on tests assessing working memory, psychomotor speed and visuoconstructional ability in using formal neurocognitive testing. There were also significant negative associations between aP-AB, S100A8/9, aNR2-AB, aGP1-AB, and lupus anticoagulant and accuracy rates on select Paediatric Automated Neuropsychological Assessment Metrics subtests (p<0.05). Over time, decline in cognitive performance was more pronounced among patients with higher NGAL and aNR2-AB levels. Combinations of serum levels of S100A8/9, S100B, NGAL, aNR2-AB and aP-AB were able to identify cSLE-NCD (sensitivity: 100%; specificity 76%) in exploratory analysis.

**Conclusions:** Select brain-reactive proteins in the blood are associated with cognitive performance and the presence of cSLE-NCD, cross-sectionally and over time. This raises the possibility that testing of these proteins may assist with the screening of cSLE-NCD.

## KEY MESSAGES

- ▶ We found levels of brain-reactive proteins associated with lower cognitive performance of children and adolescents with childhood-onset systemic lupus erythematosus (cSLE).
- ▶ These brain-reactive proteins in the blood also helped predict the course of cognitive ability in cSLE over time.
- ▶ The measurement of these brain-reactive proteins may be useful as part of the diagnostic workup of NPSLE in children.
- ▶ Complete phenotypical and unique detailed cognitive assessments as well as prospective data collection add to the validity of the results reported.

## INTRODUCTION

Children and adults with systemic lupus erythematosus are at risk of experiencing neuropsychiatric manifestations (NPSLE) involving the central or peripheral nervous systems. The mechanisms behind the wide-range of NPSLE manifestations remain poorly understood. Estimates of the prevalence of NPSLE in children range from 22% to 95%.<sup>1, 2</sup> Studies suggest that acquired clinically relevant neurocognitive deficits in childhood-onset systemic lupus erythematosus (cSLE-NCD) afflicts as many as 60% of children during the course of their disease,<sup>2-5</sup> often impairing working memory, visuoconstructional ability (VCA), attention and psychomotor speed. The criterion standard for diagnosing cSLE-NCD is formal neurocognitive testing (FNCT) using a battery of standardised tests.<sup>6</sup>

The search for early biomarkers of cSLE-NCD remains an area of active research in an effort to detect children at risk for cSLE-NCD early. Besides the use of computer-based testing<sup>7</sup> and advanced MRI



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techniques,<sup>8</sup> several brain-reactive proteins have been inconsistently associated with NPSLE.<sup>9</sup> These proteins include antibodies directed against the glutamate receptor NR2, double-stranded DNA, ribosomal P, in addition to proteins involved in immune responses such as neutrophil gelatinase associated lipocalin (NGAL), the calcium binding proteins S100B and S100A8/A9.

It is not known whether such biomarkers are relevant to the screening of cSLE-NCD.<sup>10</sup> Furthermore, the usefulness of combinations of brain-reactive proteins when measured in the blood in detecting cSLE-NCD has not been well described.

The objectives of this research were to explore the relationship between select brain-reactive proteins to serve as blood-based biomarkers for (1) identifying cSLE-NCD; and (2) predicting the course of cognitive performance of children and adolescents with cSLE over time.

## MATERIALS AND METHODS

Forty children and adolescents with cSLE were studied for 18 months. At baseline and at 18-month follow-up, blood was obtained to measure select brain-reactive proteins, and detailed assessment of cognitive performance was performed. This study was approved by the institutional review boards of both institutions and is in accordance with the ethical standards established in the 1964 Declaration of Helsinki. Prior to participation, the study was explained to each participant and their parent, and written informed consent was obtained from parents of all participants. Written assent was also obtained from participants over 11 years of age.

### Participants

Patients between the ages of 8–19 years were included, provided they fulfilled the revised American College of Rheumatology Classification Criteria for Lupus by the age of 16 years.<sup>11</sup> Patients with cSLE were excluded from participation if they had a history of comorbid conditions affecting their neurocognitive functioning prior to their diagnosis with cSLE, or if they had known structural brain abnormalities. None of the patients received specific therapy for NPSLE at the time of enrolment to the study.

### Study assessment

Besides sociodemographic data, information on clinical outcomes and medications was collected, including disease activity as measured by the Systemic Lupus Erythematosus Disease Activity Index-2000, and disease damage by the *SDI* (Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index) respectively.<sup>12 13</sup>

### Formal neurocognitive testing

For this study *FNCT* was done by trained psychometricians. A standardised neuropsychological battery suggested for

cSLE was used, with details provided elsewhere.<sup>6</sup> In brief, the battery consisted of the Wechsler Abbreviated Scale of Intelligence,<sup>14</sup> a well-validated estimate of overall intelligence (*Full Scale IQ*) and its verbal and non-verbal subcomponents; the Block Counting subtest of the Kaufman Assessment Battery for Children-II,<sup>15</sup> which measures VCA; the working memory and psychomotor speed subscales of the age-appropriate Wechsler Intelligence Scales;<sup>16 17</sup> the Wide Range Assessment of Memory and Learning-2,<sup>18</sup> from which the *Memory Screening Index* summarises an individual's ability to learn and recall new verbal and visual information; selected subtests from the Woodcock-Johnson-III Tests of Achievement<sup>19</sup> which assessed basic reading/decoding ability (*WJ-III Letter-Word Identification*) and written arithmetic skills (*WJ-III Calculation*); and the Conners' Continuous Performance Test-II<sup>20</sup> which assesses test-takers' ability to sustain attention (*CPT-II Omissions*, *CPT-II Mean Hit Reaction Time Standard Error*) and inhibit impulsive responses (*CPT-II Commissions*) during a long, boring task. Age-normed scores are available for all instruments.<sup>6</sup> The Children's Depression Inventory was completed by the participants as a self-report measure of depressive symptomatology.<sup>21</sup>

### Cognitive performance and definition of cSLE-NCD

Patient's raw test scores on each of the standardised neuropsychological tests were transformed to z-scores using published norms that corrected for age and in some cases gender and race. The z-scores of tests assessing a given cognitive domain (working memory, psychomotor speed, VCA, attention/executive functioning) were averaged to determine overall performance in each of the cognitive domains under consideration.

For normative healthy populations, domain z-scores are expected to be at a mean of 0 with a SD of 1. Notably, as ongoing cognitive development is expected during childhood and adolescence, the reference population domain z-scores will remain constant over time. Thus, any increase or decrease of a patient's z-scores indicates a relative improvement or decline in cognition over and above what would be expected, at the same age, based on population norms. A difference in z-scores of 0.5 or higher can be considered clinically important on a group level.

In the absence of a generally accepted definition for cSLE-NCD,<sup>22</sup> the following approach was taken to categorise the level of cognitive ability: Participants with at least two average cognitive domain z-scores between '−2' and '−1' were considered to have cSLE-NCD (NCD-group). Similarly, children with at least one domain z-score of '−2' or lower were classified to have cSLE-NCD. All other participants were classified to have normal cognition (noNCD-group).

### Paediatric Automated Neuropsychological Assessment Metrics

This software is a library of tests and batteries for assessing simple reaction time, attention/concentration, mental flexibility, spatial processing, cognitive-processing efficiency,

arousal/fatigue level, learning, recall and working memory.<sup>5–7</sup> Paediatric Automated Neuropsychological Assessment Metrics (Ped-ANAM) tasks are automated and various scores are generated for each test to measure patient cognitive ability. We previously showed that of the available Ped-ANAM scores, the *accuracy score*, for example, percentage of correct answers was most affected in patients with cSLE.<sup>5–7</sup>

### Biomarker assays

The choice of brain-reactive proteins considered in this study was driven by previous, albeit controversial, reports from the medical literature. All of the brain-reactive proteins were measured twice during the study, at baseline and after 18 months. Exceptions were anti-dsDNA antibodies (dsDNA-AB)<sup>23</sup> and antiglycoprotein-1 antibodies IgG (aGPI-AB) for which the results (positive or present vs negative or normal) were extracted from the medical record.

Anti-NR2 antibodies (aNR2-AB) are a subgroup of dsDNA-AB that can cross-react with an extracellular, ligand-binding domain of the N-methyl-D-aspartate receptors which are expressed on the neurons throughout the hippocampus and cortex. N-methyl-D-aspartate receptors bind the neurotransmitter glutamate and are thought to be involved in mechanisms underlying learning and memory. Elevated levels of aNR2-AB, especially in the cerebrospinal fluid are present with NPSLE.<sup>24</sup> For this study, aNR2-AB (U/mL) were measured by ELISA as previously described.<sup>25</sup>

Antiphospholipid antibodies (aPL-ABs) and aGPI-ABs have been associated with the increased incidence of white matter hyperintensities on brain MRI as well as with seizures, stroke, acute transverse myelopathy, and decreased cognitive performance in SLE.<sup>26</sup> Results of aPL-AB testing using various assays as ordered as part of clinical care were available. Specifically, results of anticardiolipin IgA and IgG, aGPI-AB IgG as well as dilute Russel Venom Viper testing were available. Given the diversity of testing methods of aPL-AB, testing was categorised as present if any of the tests was positive as per the laboratory norms.

Antiribosomal P antibodies (aP-ABs) have been associated inconsistently with psychosis, anxiety and depression. These antibodies can cause apoptosis by increasing the calcium influx and activation of caspase-3.<sup>27</sup> For this study, aP-ABs were quantified using a commercial ELISA assay (QuantaLite Ribosome P assay; Kit k981237; Inova Diagnostics; San Diego, California, USA) with values of at least 20 units/mL, representing a positive result.

The S100 proteins are members of the family of zinc-finger proteins. These proteins have been associated with tissue repair after brain injury, are secreted by astrocytes prior to entering the blood stream.<sup>28</sup> S100B has emerged as a peripheral biomarker of blood-brain barrier (BBB) permeability<sup>29–30</sup> and was measured by ELISA (#EZHS100B-33K; Human S100B; EMD Millipore Corporation, Billerica, Massachusetts, USA) with levels

presented in pg/mL; S100A8/A9 levels were determined by ELISA following the system established in our laboratory<sup>31</sup> and levels are ng/mL.

NGAL is completely absent in the normal brain but is induced in the choroid plexus of the brain following an infectious and inflammatory insult. NGAL potentiates the ability of matrix metalloproteinase 9 to increase in the BBB permeability, and NGAL levels correlate with microglia activation.<sup>32</sup> For this study NGAL (ng/mL) was measured by ELISA (KIT 036 AntibodyShop, Grussbakken, Denmark) as per the manufacturer's protocol and previously described by us.<sup>33</sup>

### Statistical analysis

Demographic and clinical characteristics were summarised by mean and SD or SE for numerical variables and frequencies (%) for categorical variables.

Numerical serum biomarkers were log transformed prior to use in statistical analysis to correct the right skewness of their distributions. Geometric means (95% CIs) were reported in the final results, after taking the exponential to the means of the log transformed variables. In association analyses, associations of numerical candidate biomarkers (log transformed) with NCD status were studied using mixed effect models, after adjusting for patient's sociodemographics and cSLE descriptors (age, race, ethnicity, annual family income, disease activity, damage, steroid dose, disease duration), with a random effect used to account for within participant-correlation caused by repeated measurements in the two visits. Similar mixed linear models were used when associations of numerical candidate biomarkers with numerical independent variables of interest were studied. Correlation coefficients were estimated through the variance-covariance matrix from such mixed linear models.<sup>34</sup>

Associations of dichotomous candidate biomarkers with NCD status as well as with other independent variables were studied using logistical regression models after adjusting for patient demographics. A generalised estimation equation method was used in computation to account for within-patient correlation in the repeated measurements.

In the prediction analyses, each of the candidate biomarkers was tested for the ability to predict the risk of cSLE-NCD using an univariate logistical regression model that accounted for within-patient correlation through a generalised estimation equation method. A receiver operating characteristic (ROC) curve was formed, based upon predicted log odds or its converted propensity score (range 0–100) to assess the performance of identifying cSLE-NCD, using individual or the combination of the candidate biomarkers under consideration. The accuracy of identifying cSLE-NCD was estimated with area under the ROC curve (AUC). In addition, a statistically 'optimal' cut-off value for propensity score, or its converted blood-based biomarker level, was identified at which its corresponding pair of sensitivity and specificity was the highest among all possible

pairs on the entire ROC curve. Using selected candidate biomarkers as predictors, a multivariate logistical regression model was also used to predict the risk of cSLE-NCD. The AUC from its ROC curve were compared with those from the univariate models, using Mann-Whitney U tests.<sup>35</sup>

Values of the AUC, sensitivity and specificity can be interpreted as outstanding, excellent, good, fair and poor performance in identifying and discriminating cSLE-NCD if they are 91–100%, 81–90%, 71–80%, 61%–70% and <60%, respectively.<sup>36</sup>

All other variables were compared between groups using t tests for interval level measurements and  $\chi^2$  tests for categorical classifications. A Pearson's correlation coefficient was used to assess relationships between continuous variables.

Statistical computations were performed using a SAS V.9.3 software (SAS, Cary, North Carolina, USA) package. p Values <0.05 were considered statistically significant.

## RESULTS

### Study population characteristics

Baseline demographics and disease information from the study participants are provided in [table 1](#). Nine of the 40 patients were diagnosed with cSLE-NCD at baseline. Follow-up data for 27 patients were available for analyses. Six of the 27 participants with follow-up information had cSLE-NCD. Four of the 27 patients developed cSLE-NCD between baseline and follow-up, while cSLE-NCD in 2 of the 27 patients was noted at baseline but had resolved at the time of follow-up. The average disease activity at enrolment was moderate, and 10 patients (25%) had SDI scores exceeding 0. However, presence of disease damage (SDI>0) was not associated with the presence of cSLE-NCD (p=0.51).

### Candidate biomarkers and cSLE-NCD status

Levels or the frequency of the candidate biomarkers in patients with cSLE-NCD (NCD-group) versus patients without cSLE-NCD (noNCD-group) are shown in [table 2](#), suggesting that elevated aP-ABs are significantly more common in the NCD-group than the noNCD group. Conversely, there was a trend towards higher serum levels of aNR2-AB in the noNCD-group versus the NCD-group. All other candidate biomarkers did not significantly differ between groups.

### Associations between candidate biomarkers and performance on cognitive tests

Patient performance in FNCT (domain z-scores) was associated with levels of aP-AB, aNR2-AB and NGAL: aP-AB levels were negatively associated with working memory, psychomotor speed and VCA but not attention/executive functioning. In addition, NGAL levels were negatively and aNR2-AB levels positively correlated with psychomotor speed ([table 3](#)).

Select concentrations of brain-reactive proteins were also significantly associated with decreased accuracy as measured by the Ped-ANAM ([table 3](#)). Similarly, presence of aGPI-AB, lupus anticoagulant (LAC) and high aP-AB levels were statistically significant predictors of inferior accuracy on select Ped -ANAM subtests.

### Relationships between the changes in brain-reactive protein levels and the change in cognitive performance over time

We then assessed the relationships between change of candidate biomarkers levels and change in cognitive performance (domain z-scores) over time. There were moderately strong negative correlations between changes in the levels of NGAL and aNR2-AB and changes in performance on tests that were completed as part of FNCT, assessing psychomotor speed and working memory, respectively. In other words, the more pronounced the decline in working memory and psychomotor speed (decrease in z-score value) the more pronounced was the increase of NGAL and aNR2-AB from baseline to 18-month follow-up.

Conversely, gain in working memory over time (increase in domain z-score value) was accompanied by increases in S100A8/9 levels over time. Likewise, increases in S100B levels over time were found to be associated with improved accuracy on select Ped-ANAM subtests over time.

### Diagnostic accuracy of brain-reactive proteins to detect cSLE-NCD

Five brain-reactive proteins were examined as predictors of cSLE-NCD using logistical regression models and ROC curves. The aGPI-AB and LAC were excluded from this analysis due to too small sample size, and dsDNA-AB due to lack of discrimination in univariate analysis.

With the exception of aP-AB levels with fair diagnostic potential (AUC>0.7), none of the other candidate biomarkers were individually accurate enough to serve as useful predictors of cSLE-NCD, given their low overall sensitivities or specificities in ROC analysis ([tables 4 and 5](#)). However, the ROC curve from the multivariate logistical model, using a set of five candidate biomarkers as predictors showed excellent accuracy in predicting and identifying cSLE-NCD with an AUC of 83.4% (95% CI 73% to 94%). A propensity score of 11 or above can identify cSLE-NCD with 100% of sensitivity and 76% of specificity in the study population.

## DISCUSSION

NPSLE continues to be a diagnosis of exclusion, given the absence of non-invasive, easily accessible and accurate diagnostic tests. This is especially true for more subtle manifestations of NPSLE, such as cognitive deficits. These are clinically difficult to appreciate, and hence require FNCT for reliable identification.



**Table 1** Demographics of study population with cSLE at enrolment

Variable*	Category	All cSLE (N=40)	NoNCD group (N=31)	NCD group (N=9)	p Value
Age	–	14.80±0.36	14.58±0.42	15.56±0.65	0.26
Female; n (%)	Yes	34 (85%)	26 (83.9%)	8 (88.9%)	0.71
Race/ethnicity n (%)	White	12 (30%)	11 (35.5%)	1 (11.1%)	0.06
	Black	18 (45%)	10 (32.3%)	8 (88.9%)	
	Hispanic	7 (17.5)	7 (22.6%)	0 (0%)	
	Asian	1 (2.5%)	1 (3.2%)	0 (0%)	
	Other	2 (5%)	2 (6.4%)	0 (0%)	
Annual family income n (%)	<\$25K	8 (20%)	5 (16.13%)	3 (33.33%)	0.12
	\$26K–\$50K	14 (35%)	9 (29.03%)	5 (55.56%)	
	\$51K–\$75K	8 (20%)	7 (22.58%)	1 (11.11%)	
	>\$75K	10 (25%)	10 (32.26%)	0 (0%)	
Disease duration (month)		23.71±3.65	23.99±4.35	22.78±6.61	0.89
Prednisone (mg/day)		19.84±3.13	15.92±2.43	33.29±10.03	0.02
Immunosuppressive drugs	Azathioprine	4 (10%)	2 (5%)	2 (22%)	0.34
	Cyclophosphamide	3 (8%)	1 (3%)	2 (22%)	
	Ciclosporin	1 (3%)	0 (0%)	1 (11%)	
	Methotrexate	1 (3%)	1 (3%)	0 (0%)	
	Mycophenolate mofetil	17 (43%)	13 (42%)	4 (44%)	
	Rituximab	1 (3%)	1 (3%)	0 (0%)	
Disease damage (SDI)†		0.40±0.13	0.35±0.14	0.56±0.34	0.52
Disease activity (SLEDAI)‡		4.88±0.69	3.90±0.48	8.22±2.34	0.007
Patient well-being§		7.73±0.25	8.00±0.27	6.78±0.52	0.04
Physician assessment of disease activity¶		2.40±0.31	2.42±0.36	2.33±0.67	0.91
Depression—Childhood Depression Index		43.75±1.22	42.65±1.27	47.56±3.03	0.09
Performance on FNCT**	Working memory	–0.29±0.11	–0.11±0.11	–0.98±0.18	<0.01
	Processing speed	–0.05±0.13	0.22±0.12	–1.11±0.18	<0.01
	Attention	0.09±0.13	0.16±0.13	–0.20±0.36	0.26
	Visuoconstructional ability (VCA)	–0.09±0.15	0.22±0.13	–1.28±0.28	<0.01

\*Values are mean±SE unless stated differently.

†Systemic Lupus Collaborative Clinics/American College of Rheumatology Damage Index.

‡Systemic Lupus Disease Activity Index 2k version; range 0–104; 0=inactive SLE.

§Measured on categorical Likert scale with 0=very poor; 10=very well.

¶Measured on categorical Likert scale with 0=inactive cSLE; 10=very active cSLE.

\*\*Formal neurocognitive testing.

cSLE, childhood-onset systemic lupus erythematosus; FNCT, Formal Neurocognitive Testing; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

Biomarkers are tests that can capture pathological processes involved in a disease. Ideally, biomarkers come from easy to collect biological samples, and are readily

quantified from rapid assays. It is also highly desirable that levels of biomarkers change with clinically relevant changes of the disease process. A biological rationale

**Table 2** Comparison of brain-reactive blood-markers with cognitive performance at baseline\*

Biomarker candidates	No-NCD-group (N=31)	NCD group (N=9)	p Value†
Anti-ds DNA antibodies % (n/N)‡	41.9% (13/31)	55.6% (5/9)	0.71
Anti-GP1 IgG antibodies positivity % (n / N)‡	40% (6/15)	0% (0/2)	1.0
Lupus anticoagulant positivity % (n / N)‡	26.7% (4/15)	0% (0/2)	1.0
Antiribosomal P antibodies positivity % (n N)‡	46.7% (14/30)	88.9% (8/9)	0.05
NR2 antibodies (U/mL)	1.0 (0.8 to 1.3)	0.7 (0.5 to 1.0)	0.08
NGAL (ng/mL)	105.5 (83.8 to 132.8)	121.8 (87.3 to 169.9)	0.66
S100B (pg/mL)	24.6 (15.1 to 40.2)	30.7 (15.7 to 59.7)	0.53
S100A8/A9 (ng/mL)	929.4 (640.9 to 1347.7)	1540.8 (827.7 to 2868.4)	0.25

\*Values are geo means (95% CI) of log-transformed biomarker concentrations in the serum, unless noted otherwise.

†p Values are from multivariate analysis adjusting for demographics and SLEDAI total score, except for the anti-dsDNA antibodies which excluded scores for these antibodies.

‡Differences in proportions were assessed for significant differences using Fisher's exact test.

NGAL, neutrophil gelatinase associated lipocalin; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

**Table 3** Correlation and association of serum biomarkers with cognitive performance

	Pearson's correlation coefficient†				OR (+SE)‡			
	S100A8/A9	S100B	NGAL	aNR2-AB	dsDNA-AB	aGP1-AB	aP-AB	LAC
Formal neurocognitive testing								
Working memory	0.14	0.02	-0.06	-0.23	0.023±0.46	-0.324±0.46	-1.281±0.61*	0.297±0.53
Processing speed	-0.02	0.02	-0.27*	0.26*	0.086±0.34	1.902±1.24	-0.955±0.45*	-0.031±0.64
Attention	-0.03	-0.05	-0.21	0.12	0.095±0.30	0.142±0.31	-0.487±0.31	-0.151±0.29
VCA	-0.04	-0.12	-0.05	0.18	-0.139±0.31	-0.074±0.38	-1.399±0.46**	0.137±0.46
Ped-ANAM								
CDD	0.00	-0.20	0.00	0.02	0.012±0.02	-0.068±0.04*	-0.011±0.02	-0.067±0.03**
CS	-0.14	-0.03	0.19	-0.16	0.014±0.04	-0.317±0.15*	-0.019±0.04	-0.146±0.13
CPT	0.12	-0.05	0.14	0.14	-0.010±0.01	-0.031±0.02	-0.020±0.01*	0.013±0.02
Logical	-0.09	-0.09	0.17	-0.04	0.021±0.04	-0.198±0.11*	0.001±0.03	-0.099±0.09
M2S	-0.01	-0.11	0.19	0.11	-0.013±0.01	-0.042±0.03	-0.017±0.02	-0.021±0.02
MG	-0.09	0.10	0.16	-0.21*	0.029±0.03	0.034±0.09	-0.006±0.02	-0.011±0.06
Math	-0.28*	-0.05	0.06	-0.19	0.009±0.04	-0.092±0.06	0.003±0.04	-0.231±0.10*
Spatial	0.07	0.00	0.14	-0.09	-0.034±0.03	-0.064±0.08	-0.024±0.03	0.000±0.08
Memory	0.08	0.10	0.21	-0.14	-0.034±0.04	-0.064±0.04	-0.023±0.04	-0.032±0.04
Overall	0.06	-0.01	0.20	-0.01	0.000±0.00	-0.017±0.01*	-0.003±0.00	-0.009±0.01

\*p&lt;0.05.

\*\*p&lt;0.01.

†A correlation coefficient was estimated from the variance-covariance matrix from a mixed effect model after pooling data from two visits and adjusting for demographics.

‡A log (OR) was estimated from a logistic regression model after pooling data from two visits and adjusting for demographics.

Accuracy, percentage correctly answered questions per Ped-ANAM subtest; Ped-ANAM Subtests: CDD, Code Substitution Delayed; CS: Code Substitution; CPT, Continuous Performance Test; Logical, Logical Relations; M2S, Matching to Sample; MG, Matching Grids; Math, Mathematical Processing; Spatial, Spatial Processing; Memory, Sternberg Memory Search.

ds DNA-AB, anti-dsDNA antibodies; aGP1-AB, antiglycoprotein-1 antibodies; aNR2-AB, antibodies to NR2 glutamate receptor; aP-AB, antiribosomal P antibodies; LAC, lupus anticoagulant; NGAL, neutrophil gelatinase associated lipocalin; Ped-ANAM, Paediatric Automated Neuropsychological Assessment Metrics; VCA, visuoperceptual ability.

**Table 4** Correlations of changes of serum biomarkers versus changes of cognitive performance over time (N=27)†

	S100A8/9	S100B	NGAL	aNR2-AB
Formal neurocognitive testing				
Working memory	0.44*	0.07	0.26	-0.59**
Processing speed	-0.06	0.03	-0.46*	0.22
Attention	0.16	0.16	-0.16	0.00
VCA	-0.02	0.12	-0.11	0.12
Ped-ANAM				
CDD	0.15	0.48*	0.10	0.05
CS	0.18	0.41*	0.28	-0.12
CPT	0.24	0.07	-0.13	-0.01
Logical	0.19	0.32	0.13	0.12
M2S	0.06	0.00	0.01	0.38*
MG	0.15	0.30	0.33	-0.21
Math	0.10	0.36	0.08	0.16
Spatial	0.40*	0.44*	0.30	-0.25
Memory	-0.01	0.44*	0.08	-0.17
Overall	0.29	0.46*	0.15	0.06

\*p Value &lt;0.05. \*\*p Value &lt;0.01.

†Values are Pearson's correlation coefficients.

aNR2-AB, antibodies to NR2 glutamate receptor; NGAL, neutrophil gelatinase associated lipocalin; Ped-ANAM, Paediatric Automated Neuropsychological Assessment Metrics; Ped-ANAM Subtests: CDD, Code Substitution Delayed; CS: Code Substitution; CPT, Continuous Performance Test; Logical, logical relations; M2S, Matching to Sample; MG, matching grids; Math, mathematical processing; Spatial, spatial processing; Memory, Sternberg Memory Search; VCA, visuoperceptual ability.

was present, based on animal and human studies, for all the brain-reactive proteins considered in this study. In this pilot study we explored a panel of brain-reactive antibodies for their ability to serve as biomarkers for cSLE-NCD. We found select blood-based proteins associated with the presence of cSLE-NCD and cognitive ability and its course. Based on this, we propose that the brain-reactive proteins considered in this study are candidate biomarkers of cSLE-NCD.

While previous studies have shown that these proteins are present particularly in the cerebrospinal fluid of patients with NPSLE,<sup>23 25 37-40</sup> lumbar punctures are rarely used in a paediatric clinical setting for the screening of cSLE-NCD due to procedural cost, patient discomfort and medical risks. In contrast, blood sampling can be easily done as part of routine clinical care.

We obtained detailed phenotypical information of the study participants including a thorough and standardised assessment of cognitive ability. This allowed us to newly demonstrate the differential association of select candidate biomarkers with distinct cognitive domains, cross-sectionally and over time. We hypothesise that the well described phenotypical variability of cSLE-NCD, in terms of its severity and cognitive skills may be the reflection of various cSLE-associated antibodies and their differential time-dependent impact on the brain.

In this pilot study we found aP-AB more common among patients with versus without cSLE-NCD,

Table 5 Summary of univariate and multivariate logistical models and diagnostic accuracy of identifying NCD\*

Serum biomarkers	MU1	MU2	MU3	MU4	MU5	MM1
Intercept	-4.11±1.96	-2.61±0.96	-4.90±2.84	-1.41±0.39	-2.60±1.03	0.02±0.31
S100A8/9	0.40±0.29					0.62±0.43
S100B		0.41±0.28				1.69±1.16
NGAL			0.78±0.57			-0.94±0.68
aNR2-AB				-0.81±0.52		3.35±1.11
aP-AB					2.03±1.10	1.82±0.82
AUC (95% CI) in %	69.0 (53.8 to 84.3)	62.8 (46.0 to 79.7)	56.5 (39.1 to 74.0)	62.5 (47.9 to 77.0)	70.3 (59.0 to 81.6)	83.4 (73.3 to 93.5)
p Value†	0.023	0.004	0.001	0.002	0.004	Reference
Cut-off value‡	S100A8/9>850	S100B>30	NGAL>240	aNR2-AB<1	aP-AB≥1	>11\$
Sensitivity (%)	85.7	57.1	26.7	86.7	86.7	100.0
Specificity (%)	51.0	70.2	92.0	51.0	54.0	76

\*A MU model uses one SBM as the single predictor in the logistical model and the MM1 uses all listed predictors in the model.

†p Value is used to compare AUCs between a MU model and MM1 model using non-parametric Mann-Whitney U tests.

‡The cut-off is corresponded to a 'best' pair of sensitivity and specificity which the average is the largest among all possible pairs on the receiver operating characteristic curve.

\$The value represents a cut-off of propensity score from the multivariate logistical model MM1. The propensity score in this model is calculated in two steps. In step 1, a raw score or r-score is

calculated using the r-score=0.62xS100A8+15+1.7xS100B-0.9xNGAL+3.4xNR2+1.8xRibosomal-P+0.8xDs-DNA+0.02. In the next step, the r-score is converted into the propensity score or

p-score using p-scores=100xexp(r-score)/(1+exp(r-score)).

aP-AB, antiribosomal P antibodies; AUC, area under the receiver operating characteristic curve; NGAL, neutrophil gelatinase associated lipocalin; aNR2-AB, antibodies to NR2 glutamate receptor.

significantly associated with low performance in several cognitive domains and over 85% sensitive for identifying cSLE-NCD. These findings are in line with the results of some previous studies.<sup>40 41</sup>

It has been postulated that a global or regional break of the BBB is necessary for antibodies to cross and enter the brain.<sup>42-44</sup> S100B is now a well-recognised biomarker of decreased BBB integrity, and is often used in the setting of traumatic brain injury and infection; S100A8/9 is considered a marker of inflammatory disease states. We found S100B only weakly negatively correlated with measures of cognitive functioning cross-sectionally and, other than expected, even positively associated with cognitive ability over time, on FNCT and select Ped-ANAM subtests. However, our findings are in line with a recent study that suggests S100B does not help differentiating between the presence and absence of various NPSLE syndromes in children.<sup>45</sup> We consider the weak positive associations between S100A8/A9 with the change in Ped-ANAM performance over time to be clinically irrelevant. However, based on our multivariate analysis, S100 proteins may still contribute to the identification of cSLE-NCD, hence further study seems warranted to assess the value of S100 measurement in the setting of cSLE-NCD.

Besides being a biomarker of lupus nephritis when measured in the urine, plasma NGAL serves as biomarker of vascular brain injury and paediatric brain tumours.<sup>46</sup> NGAL prolongs the half-life of matrix metalloproteinase 6, a peptide that can weaken the integrity of the BBB. In this pilot evaluation we found high NGAL levels no different between groups of patients with and without cSLE-NCD at baseline. However, increasing NGAL levels were associated with worsening psychomotor speed over time. To the best of our knowledge, we are the first to report an association between NGAL and diminished and declining psychomotor speed in children with cSLE.

aNR2-ABs have been reported to promote degenerative changes in the hippocampus and lead to neuronal death.<sup>42</sup> While there were no differences in aNR2-AB levels at baseline between the NCD-group and the noNCD-groups, we found increasing aNR2-AB levels negatively associated with decline in working memory.

There are a considerable number of reports that aPL-ABs are associated with NPSLE. We also found some associations between LAC and aGPI-AB with select Ped-ANAM subtests but not with the results of FNCT. Lack of strong associations with cSLE-NCD might be due to the fact that laboratory testing for LAC and aGPI-AB was done using various laboratory tests. The same might hold true for the previously reported association between dsDNA-AB and NPSLE which we could not verify.

Given the well-described phenotypical variability of NPSLE and cSLE-NCD, it is likely that several pathological pathways are involved, making a single cSLE-NCD biomarker unlikely. This notion is in line with our findings of a panel of brain-reactive proteins being

differentially associated with various cognitive skills and their changes over time. Interestingly, our exploratory multivariate regression model includes biomarkers that are involved in weakening the BBB and those shown to be involved in the damage of brain tissues.

Limitations of this pilot study include its relatively small sample size, especially as it pertains to follow-up data. However, we feel that the results of our investigations are nonetheless important, given the excellent phenotypical information available to us, which included the results for FNCT and the Ped-ANAM.

We are well-aware that cerebrospinal fluid evaluation, rather than blood testing, of the brain-reactive proteins included in this study would likely yield a clearer understanding of relationships between the cSLE-NCD and cognitive performance. However, such knowledge cannot be exploited effectively to monitor children and adolescents with cSLE, given the invasive nature and cost of serial lumbar punctures.

Hence, discovery and longitudinal valuation of blood-based biomarkers appears warranted. Such biomarkers, alone or combined with advanced imaging and performance-based screening test are expected to facilitate the early diagnosis and monitoring of cSLE-NCD in the future. If the findings of our pilot study are confirmed in larger patient populations then blood-based biomarkers may become readily available to support the screening for cSLE-NCD and NPSLE at large.

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