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On cerebral lupus: from pathogenesis to clinical outcomes

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ON CEREBRAL LUPUS

– FROM PATHOGENESIS TO CLINICAL OUTCOMES –

CÉSAR MAGRO CHECA

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CÉSAR MAGRO CHECA

2019

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ON CEREBRAL LUPUS

– FROM PATHOGENESIS TO CLINICAL OUTCOMES –

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To my parents
To Sunna

TABLE OF CONTENTS

Chapter 1 – General Introduction	11
<i>Adapted from Drugs. 2016; 76: 459-83.</i>	
PART 1: LABORATORY BIOMARKERS	29
Chapter 2 – C1q deficiency and neuropsychiatric systemic lupus erythematosus	31
<i>Front Immunol. 2016; 7: 647.</i>	
Chapter 3 – Complement levels and anti-C1q autoantibodies in patients with neuropsychiatric systemic lupus erythematosus.	49
<i>Lupus. 2016; 25: 878-88.</i>	
Chapter 4 – Cluster analysis of an array of autoantibodies in neuropsychiatric systemic lupus erythematosus.	67
<i>J Rheumatol. 2014; 41: 1720-1.</i>	
PART 2: NEUROIMAGING BIOMARKERS	75
Chapter 5 – Are serum autoantibodies associated with brain changes in systemic lupus erythematosus? - MRI data from the Leiden NP-SLE cohort.	77
<i>Lupus. 2019; 28:94-103.</i>	
Chapter 6 – Changes in white matter microstructure suggest inflammatory origin of neuropsychiatric systemic lupus erythematosus.	103
<i>Arthritis Rheumatol. 2016; 68:1945-54.</i>	
Chapter 7 – Glial and axonal changes in systemic lupus erythematosus measured with diffusion of intracellular metabolites.	123
<i>Brain. 2016; 139: 1447-57.</i>	
PART3:IMPROVINGATTRIBUTIONOFNEUROPSYCHIATRICMANIFESTATIONS IN SYSTEMIC LUPUS ERYTHEMATOSUS	143
Chapter 8 – Value of multidisciplinary re-assessment in attribution of neuropsychiatric events to systemic lupus erythematosus.	145
<i>Rheumatology (Oxford). 2017; 56: 1676-83.</i>	
Chapter 9 – Outcomes of neuropsychiatric events in systemic lupus erythematosus based on clinical phenotypes; prospective data from the Leiden NP-SLE-cohort.	163
<i>Lupus. 2017; 26: 543-51.</i>	

Chapter 10 – Summary and conclusions	181
<i>Adapted from Front Med. 2018; 4;5:340. Adapted from Drugs. 2016; 76: 459-83.</i>	
Chapter 11 – Samenvatting en conclusies	197
APPENDICES	207
List of publications	208
Curriculum Vitae	212
Acknowledgements	213

1

GENERAL INTRODUCTION

Adapted from Drugs. 2016; 76: 459-83.

GENERAL INTRODUCTION

Systemic lupus erythematosus (SLE) is the archetype of autoimmune diseases. It is characterized by global loss of tolerance to self-antigens and activation of autoreactive T and B cells leading to the production of a wide range of autoantibodies and formation of immune complexes, which results in tissue inflammation and organ dysfunction. SLE occurs mainly in young women of childbearing age and has an estimated prevalence of 1-2:1000 in high risk groups such as Afro-American, Asian and Hispanic. Clinically, SLE manifests with a broad spectrum of clinical presentations which usually follow a waxing and waning course.(1) Central and peripheral nervous system involvement lead to a nonspecific and heterogeneous group of neuropsychiatric (NP) manifestations known with the generic definition neuropsychiatric systemic lupus erythematosus (NP-SLE) (Table 1).(2) These NP manifestations usually occur early in the course of SLE and in 39–50 % of patients as the presenting symptom of SLE.(3) A recent meta-analysis pooling all available studies and including 5057 SLE patients showed that the prevalence of NP-SLE was 44.5 % in prospective studies versus 17.6 % in retrospective studies.(4) NP-SLE is a severe complication of SLE that contributes considerably to quality of life, morbidity and mortality.(5) A tenfold increase in mortality rate in NP-SLE compared with the general population has been reported.(6)

Table 1. Neuropsychiatric syndromes according to the American College of Rheumatology

	Central Nervous System	Peripheral Nervous System
Neurological syndromes	Focal	1. Aseptic meningitis
		2. Cerebrovascular disease
		3. Demyelinating syndrome
		4. Headache
		5. Movement disorder
		6. Myelopathy
		7. Seizure disorders
Psychiatric syndromes	Diffuse	8. Acute confusional state
		9. Anxiety disorder
		10. Cognitive dysfunction
		11. Mood disorder
		12. Psychosis
		13. Guillain-Barré
		14. Autonomic disorder
		15. Mononeuropathy single/multiplex
		16. Myasthenia Gravis
		17. Cranial neuropathy
		18. Plexopathy
		19. Polyneuropathy

From pathogenic mechanisms to pathophysiological changes in NP-SLE

The exact pathogenic processes that lead to damage or dysfunction in the nervous system of SLE patients resulting in pathophysiological changes and subsequently in clinical manifestations remain poorly understood. Dozens of risk factors indicative of putative mechanisms have been proposed as candidates in the genesis of nervous system involvement in SLE (**Supplementary Table 1**).⁽²⁾ They may be grouped into two separate main pathogenic mechanisms, which are thought to lead to the two described underlying pathophysiologic processes in NP-SLE (**Figure 1**):^(7,8)

1. Thrombotic/ischemic: autoantibodies (e.g. antiphospholipid antibodies), immune complexes, complement deposition, leukoagglutination, and accelerated atherosclerosis leading to vascular injury and occlusion characterized by a thrombotic process of the large and small (micro-angiopathy) intracranial vessels.
2. Inflammatory: autoantibodies or inflammatory mediators with either a disrupted blood–brain barrier (BBB) or intrathecal formation of immune complexes leading to neuronal dysfunction and inflammation which may be induced directly by these mediators or indirectly through activation of other neural cells such as microglia.

In a considerable proportion of NP-SLE patients, both pathophysiologic changes have been reported to coexist and to manifest as a wide heterogeneous group of NP features.⁽⁸⁾

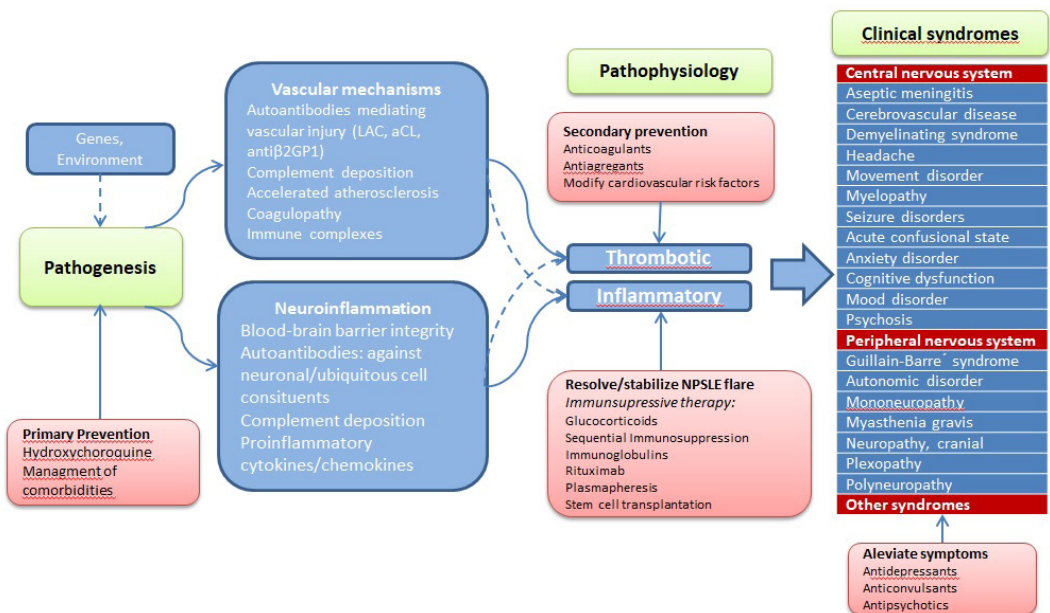


Figure 1. NP-SLE diagram: from bench to bedside. aCL anticardiolipin antibodies, b2GP I b2-glycoprotein I, LAC lupus anticoagulant, NP-SLE neuropsychiatric systemic lupus erythematosus

Autoantibodies

Autoantibodies are thought to play a crucial role in NP-SLE pathogenesis. Brain tissue-reactive antibodies in NP-SLE can be synthesized in the CNS or in peripheral organs (lymph nodes and bone marrow). In the latter, these autoantibodies must pass through the BBB of SLE patients to exert an effect upon neurons. Why and how the integrity of the BBB is compromised and which antibodies pass through the barrier and lead to damage/dysfunction is not fully understood.(9) The majority of studies simply report the higher presence of a certain antibody in serum and/or cerebrospinal fluid (CSF) of NP-SLE patients, and none of these findings have remained as reproducible as to become a specific biomarker for NP-SLE or any individual NP manifestation. Although no specific autoantibodies have been identified, several studies have confirmed an important association between antiphospholipid antibodies (aPL), especially lupus anticoagulant (LAC), and anti-ribosomal P antibodies, and cerebrovascular disease and psychosis, respectively.(10) In the last years, new multiplex immunoproteomic approaches for the detection of autoantibodies have emerged. These techniques have a higher sensitivity and broader dynamic range than traditional systems. The use of these arrays may help to uncover antibody clusters that may distinguish between NP manifestations related and not related to SLE.(11)

Complement cascade

Complement components are known to play a major and complex role in SLE (**Figure 2**). While genetic deficiencies of many classical pathway components (C1q, C1r, C1s, C2 and C4) are strongly associated with the development of SLE, paradoxically, complement contributes to the inflammatory tissue destruction observed in SLE.(12)

Complement is also well known to play a role in normal brain development and to contribute to the pathology of inflammatory central nervous system (CNS) and neurodegenerative diseases.(13) Based on mice models, complement has been proposed as one of the multiple participants in the pathogenesis of NP-SLE.(14) Complement plays an important role in synaptic pruning, BBB alteration, vasculopathy and accelerated atherosclerosis. (14,15) Recent discoveries implicate both the classical complement cascade and interferon α as major pathways used by microglia for synaptic pruning. In SLE, chronic peripheral inflammation may play a role in the aberrant activation of microglia and subsequently stimulate synapse loss, tagging inappropriate synaptic connections between neurons.(16) Furthermore, complement has been proposed as an important modulator of the integrity of the BBB. The BBB is a network of endothelial cells and pericyte and astrocyte projections that regulates the entry of soluble molecules and cells into the brain parenchyma. The disruption of the BBB integrity may permit the influx of neuropathic antibodies which may target synapses for engulfment by microglia.(9) Studies in MRL/lpr mice, accurately reflecting human NP-

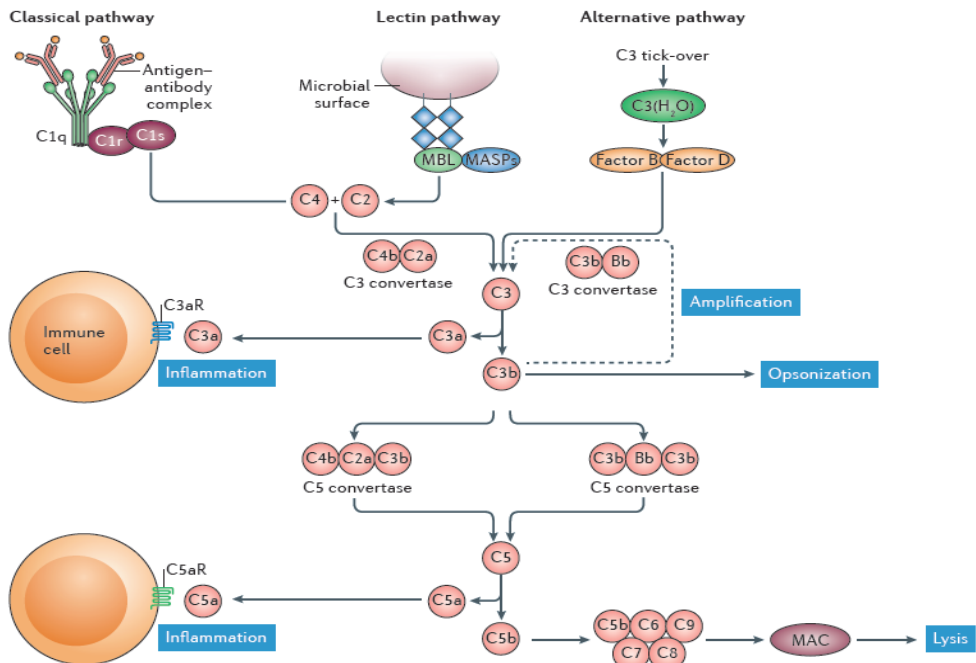


Figure 2. The complement cascade. The complement system can be activated via one of three pathways: the classical pathway, the lectin pathway and the alternative pathway. These pathways are activated following binding of the recognition molecule C1q to ligands such as immune complexes (classical pathway) and mannose-binding lectin (lectin pathway). Alternative pathway is initiated spontaneously or following binding of properdin. Activation of the complement cascade facilitates opsonisation (via C3b), promotes inflammation by attraction of immune cells (via C5a) and damage of pathogen cells (via C5b-C9 membrane attack complex (MAC)). Printed with permission from Noris M, et al. *Nat Rev Nephrol.* 2012;8:622–633.

SLE, have shown the importance of the alternative complement cascade in BBB disruption. Complement component C5 has been reported to play a role in the maintenance of the BBB in mice.⁽¹⁷⁾ Selective inhibition of C5aR alleviated CNS lupus.⁽¹⁸⁾ Furthermore, complement plays a role in microvascular injury. Mice deficient in C3 and C5 components are resistant to enhanced thrombosis and endothelial cell activation induced by aPL antibodies, indicating the important role of alternative pathway complement activation on aPL antibody-mediated thrombogenesis.⁽¹⁹⁾ Besides all the previous arguments to think about a potential role of complement in NP-SLE pathogenesis, studies analysing complement in human NP-SLE are very scarce.

Neuroimaging

Magnetic resonance imaging (MRI) is the neuroimaging technique of choice in NP-SLE and remains the neuroimaging test used in clinical practice. This technique is able to localize abnormalities in the brain and spine, allowing the identification of lesions associated with

NP-SLE (e.g. infarcts) and many differential disorders (e.g. tumours or infections). However, MRI is nonspecific and in a significant number of patients no abnormalities or only white matter hyperintensities are found, independently of the NP-SLE syndrome and severity.(20) Other advanced neuroimaging methods based on MRI have shown to capture the nature of tissue microstructural damage related to SLE, and thus postulated to contribute to the understanding of the pathophysiological changes:

Magnetization transfer imaging (MTI): this technique can play an important role in assessing the disease burden by applying magnetization transfer ratio (MTR) histogram analysis on whole or segmented (ie. grey matter or white matter) brain tissue **(Figure 3)**.(21,22) This technique reflects in a quantitative way the integrity of macromolecular structures that exchange magnetization with the surrounding water and is sensitive to macroscopic an microscopic abnormalities. Among all MTR-derived parameters, the MTR histogram peak height (MTR-HPH) is the most informative in patients without explanatory MRI findings in NP-SLE.(23) In preliminary investigations, lower MTR-HPH has been observed in NP-SLE patients. This value has been found to be correlated with neurocognitive impairment and with the clinical status of NP-SLE patients.(24,25)

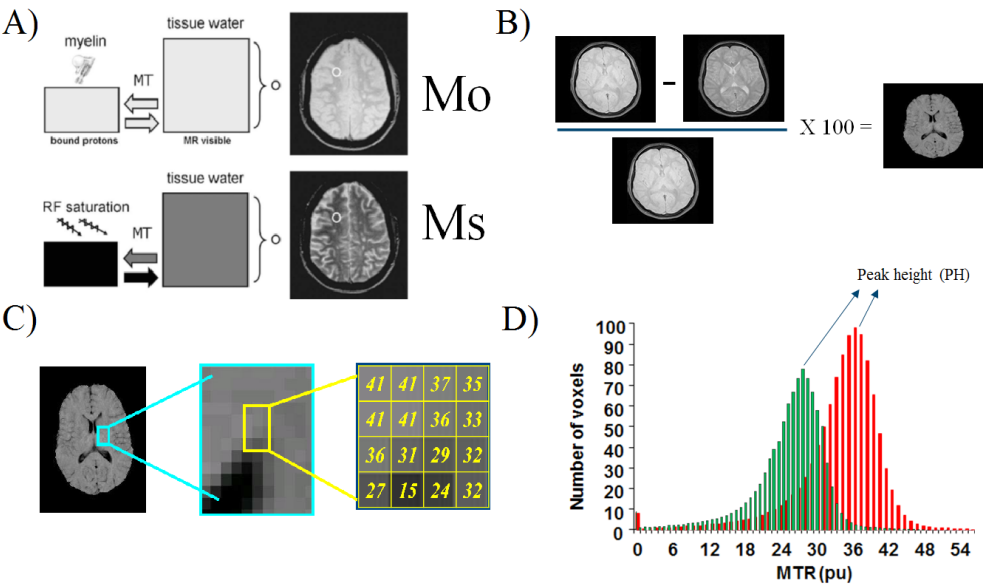


Figure 3. Basis of Magnetization Transfer Imaging. **A)** This technique is based on the application of off-resonance radiofrequency pulses. M0: proton density image or intensity of voxels without saturation, Ms: Bound protons or intensity of voxels saturated **B)** Measurement of signal intensity with and without the application of these pulses allows the calculation of an index called the magnetization transfer ratio (MTR) which is defined as $(M_0 - M_s) / M_0 \times 100\%$ **C)** MTR histogram: this technique takes a ratio of the two images on a voxel-by-voxel basis (brain pixels). **D)** The histogram peak height (HPH), a MTR histogram-derived measure, accounts for the proportion of brain pixels at the most common MTR value. Figure 3A partially adapted from Grossman RI et al. Radiographics. 1994 ;14(2):279-90 (21).

Proton Magnetic resonance spectroscopy (1H-MRS): this technique is a non-invasive test that permits chemically specific, non-invasive measurements of the concentration of neuronal metabolites such as N-acetylaspartate (NAA), myoinositol (MI), choline-containing compounds (Cho), total creatine (tCr), glutamine (Gln) and glutamate (Glu).(26)

This technique has been used in SLE studies where differences in the concentrations of several metabolites (relative to tCr) have been reported.(27) Lower NAA and higher Cho and MI levels have been reported in SLE and NP-SLE patients when compared to healthy controls. Furthermore, lower NAA changes in NP-SLE patients when compared with SLE and in SLE with high disease activity when compared with low activity were found.(28,29)

Diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI): this technique measures the microscopic motion of water protons. Differences in the magnitude of diffusion of water molecules in the brain, which depends on several known and unknown factors, can be translated into image contrast and quantitative brain maps. The rate of diffusion in living systems is referred to as the apparent diffusion coefficient (ADC) or mean diffusivity (MD). Molecular movement in tissues such as white matter is not the same in all directions, which is known as anisotropy. DTI analyses the three-dimensional shape of the diffusion. With this technique white matter fiber tracts can be reconstructed by combining the magnitude and directionality information of this anisotropic diffusion.(30) The magnitude is represented by the MD while the directionality of water diffusion is represented by the fractional anisotropy (FA). Moreover, the rate of diffusion in the principal direction and the perpendicular direction to the white matter tract are called axial diffusivity and radial diffusivity (RD), respectively. All these parameters provide sensitive, but nonspecific measures of microstructural changes in brain tissue and are typically assessed in clinical studies by region-of-interest or voxel-based analysis.(31) DWI studies showed significant increase in ADC of the NP-SLE patients compared to healthy controls.(32,33)

Some studies have shown that in NP-SLE, there is a relationship between MTR-HPHs and neurochemical findings revealed with 1H-MRS.(23) However, the relationship between anatomical distribution and microstructural damage using different neuroimaging techniques have been so far scarcely studied. Furthermore, besides the promising results, the clinical relevance of these tests in both group and individual SLE patients requires further evidence and validation.

Attribution of NP manifestations to SLE: a challenge for the clinician

No laboratory or radiological biomarker nor other formal system exists for establishing a diagnosis and guiding therapy decisions in NP-SLE. In SLE, < 40 % of the NP symptoms will be attributed to SLE-induced nervous system damage. In the remaining cases, other

causes (i.e. therapy, primary NP disorders) will better explain these symptoms. Given the absence of a gold standard in the diagnostic approach, NP-SLE remains a diagnosis per exclusionem and is mainly based on expert opinion. In all patients, there is an obligation to first strictly exclude other potential causes such as infection, coincidental disease processes, metabolic abnormalities, or drug side effects. In SLE patients presenting with unexplained NP symptoms or signs suggestive of NP disease, the first step would be to evaluate and characterize the NP symptoms, similarly to patients without SLE.(34)

In 1999, the American College of Rheumatology (ACR) published a set of NP-SLE case definitions, including 12 CNS and 7 peripheral nervous system manifestations.(35) Although this standardized approach to categorize NP events in SLE patients has improved the description and classification of NP-SLE in clinical studies, its usefulness in clinical practice is limited. Several CNS syndromes (headache, anxiety, mood disorder, and mild cognitive disorder) included in these definitions are nonspecific. Although frequently seen in SLE patients, these syndromes may only be attributed to SLE in selected cases within an appropriate clinical context.(36,37) Bortoluzzi et al. proposed an algorithm that may assist the rheumatologist in the attribution of NP events to SLE, however this method should be not used as a substitute of expert physician judgement.(38) In a clinical setting, NP-SLE can also be classified according to the suspected underlying pathophysiologic process; this phenotypic diagnosis differs between ischemic and inflammatory NP-SLE.(8) We have previously recommended multidisciplinary expert consensus after a standardized assessment as the reference standard for diagnosing and classifying NP-SLE. In some cases, the diagnosis will be inevitably presumptive; therefore, it has been proposed that patients must be prospectively followed and reanalysed to avoid misclassification.(8) The value of the re-assessment of these patients has never been addressed.

Therapy and outcome of NP manifestations in SLE patients

Specific NP-SLE therapy remains relatively empirical due to the scarcity of controlled trials. To date, only one randomized controlled treatment trial in NP-SLE has been undertaken.(39) In the acute setting, management of these patients does not differ from other non-SLE subjects presenting with the same NP manifestation. Afterwards, an individualized therapeutic strategy, depending on the presenting manifestation and severity of symptoms, must be started. Several therapeutic strategies report benefits in different aspects of NP-SLE management: primary prevention, resolution and stabilization of acute symptoms, maintenance therapy, and secondary prevention. In clinical practice, treatment of a phenotypic diagnosis is preferred; depending on the suspected underlying pathophysiological process, therapy will be directed at inflammation or at prevention of ischemic events.(8) Manifestations that are thought to reflect an immune-inflammatory state or, in the presence of generalized

lupus activity, initiation of immunosuppressive therapy is warranted (corticosteroids alone or in combination with another immunosuppressant), with the main objective of resolving/stabilizing symptoms. On the other hand, anticoagulation and antiplatelet agents are the mainstay of secondary prevention after ischemic NP-SLE, especially in the presence of aPL (**Figure 1**).⁽³⁴⁾ In the Leiden NP-SLE clinic, a treatment algorithm was developed based on available evidence and our own expertise, although the treatment of NP-SLE patients must always be individually tailor made (**Supplementary Figure 1**).

The clinical outcome of NP events presenting in SLE, either related or non-related to the disease, has been scarcely studied. Two previous investigations, presenting in the large inception Systemic Lupus International Collaborating Clinics (SLICC) cohort, found that the outcome in NP-SLE events was more favourable than in non-NP-SLE events.^(3,5) The occurrence of NP events in SLE patients, independent of their aetiology, has been associated with a considerable comorbidity, resulting in marked adverse repercussions on health-related quality of life (HRQoL).⁽³⁾ Among all the available tools for measuring HRQoL, the 36-item Short Form Health Survey (SF-36) is a valid and reliable tool to identify the effect of SLE in the physical, mental and social domains of these patients.⁽⁴⁰⁾ SF-36 has been associated with the clinical outcome of NP events in SLE patients.⁽⁴¹⁾ However, it is unknown how a certain pathophysiological mechanism of NP-SLE may impact clinical outcome and SF-36 domains change over time.

The Leiden NP-SLE clinic

All patients included in this thesis are part of the Leiden NP-SLE cohort.

Our institution, the Leiden University Medical Center (LUMC) serves as a national referral center for NP-SLE in the Netherlands. The Leiden NP-SLE clinic was established in September 2007 to evaluate SLE patients presenting with NP manifestations in a standardized, multidisciplinary and prospective way. Between September 2007 and September 2017, a total of 480 consecutive patients who were suspected by a referral doctor of having NP-SLE were evaluated. All patients included in the Leiden NP-SLE cohort were admitted for a 1-day program where they were assessed by specialist in rheumatology, neurology, psychiatry and vascular medicine. Extensive laboratory tests, 3-tesla MRI of the brain including magnetic transfer imaging (MTI) and neuropsychological testing were routinely performed. Additional cerebrospinal fluid analysis, electromyogram, electroencephalogram, evoked potentials, MRI of the spine or MR angiography were performed when indicated.^(8,42)

Table 2. Procedure in evaluation of patients in the Leiden NP-SLE clinic

Start	Inclusion		2 Weeks	≥ 3 Months
Referral by treating physician	Evaluation by: Rheumatologist Neurologist Psychiatrist Vascular internist Neuropsychologist	Additional tests: MRI brain, MTI, RS fMRI, blood tests, urine tests, neuropsychological tests, SF-36, HADS, DES, NPI	Consensus meeting: Rheumatologist Neurologist Psychiatrist Vascular internist Neuropsychologist Radiologist	Follow-up: Rheumatologist Neurologist Psychiatrist Vascular internist Neuropsychologist MRI brain

DES: Dissociation Experience Scale, HADS: Hospital Anxiety and Depression Scale, MRI: magnetic resonance imaging; MTI: magnetic transfer imaging, NPI: Neuropsychiatric Inventory, RS fMRI: resting state functional MRI, SF-36: Short Form-36.

The final attribution of NP events to SLE or other aetiologies was made by multidisciplinary consensus of all participating specialists and after the evaluation of all serological, neuroimaging and neuropsychological assessments. Specialists met in a 2-weekly scheduled meeting to discuss the patients and determine the origin of NP events. During the consensus meeting the next aspects were taken into account: objective confirmation of symptoms (assessed to standard of care of the appropriate medical specialty); exclusion of other aetiology explaining these symptoms; NP event possibly caused by SLE. Furthermore, when NP complaints were not clearly explained by other disease (e.g. Parkinson's disease, schizophrenia), a re-assessment of the patient took place after 3-18 months to evaluate the evolution over time and the response to therapy. At this point, all these patients were assessed by the same specialist and underwent the same multidisciplinary assessment.

Table 2 offers an outline of patient assessment.(8)

AIMS AND OUTLINE OF THE THESIS

This thesis has the next main aims:

1. To analyse the association between novel laboratory or neuroimaging findings and the different pathophysiological changes (inflammatory and ischemic) or clinical manifestations in NP-SLE.
2. To analyse longitudinally the attribution of NP manifestations to SLE and the outcome of these events.

The ultimate goal is to improve the diagnosis and treatment of NP manifestations presenting in patients with SLE in the clinical setting.

This thesis consists of three main parts:

In **part I**, several potential serum laboratory biomarkers for NP-SLE are analysed, particularly the complement cascade and serum autoantibodies. So far, deficiencies of early classical pathway complement system components (C1q, C1r, C1s, C2 or C4), are the strongest known disease susceptibility genes for the development of SLE in humans.(43) NP-SLE has only been described in C1q deficiency. In **Chapter 2**, we analyze the clinical and molecular basis of a C1q deficiency patient presenting with severe NP-SLE and make a review of the literature. In **Chapter 3**, we describe the associations between serum complement cascade components, anti-C1q and C1q immune-complexes and the clinical NP-SLE manifestations. The association between these NP-SLE manifestations and clusters of serum autoantibodies is analysed in **Chapter 4** using multiplex immunoassay for the simultaneous detection of several autoantibodies, a technique thought to increase specificity of biomarkers.

In **part II**, we assess the role of both qualitative and quantitative neuroimaging techniques in the identification of the different underlying pathophysiological processes (inflammatory or ischemic) and therefore its usefulness as diagnostic biomarkers in NP-SLE. An unresolved problem is the role of direct auto-antibody-mediated brain damage in SLE. MRI has been demonstrated to be a good surrogate marker of pathology in NP-SLE. Furthermore several MRI phenotypes have been proposed in the past.(20,44) In **Chapter 5** we analyse the relationship between the underlying autoimmune profile measuring individual and cumulative serum autoantibodies and SLE-associated brain abnormalities found on 3-Tesla MRI. Moreover, we study in which magnitude these MRI findings would be driven by the classical cardiovascular disease risk factors or other SLE-related factors. Quantitative MRI techniques are known to be useful to detect cerebral abnormalities in normal appearing brain tissue on conventional MRI. MTI has been successfully applied to small groups of SLE and NP-SLE patients. It remains unclear whether these changes highlight an underlying

pathophysiological process, if they are linked to the clinical status or to a specific NP-SLE syndrome. In **Chapter 6**, white matter (WM) and grey matter (GM) MTR-HPHs are prospectively assessed in a group of SLE patients presenting with NP manifestations either related or unrelated to SLE. Furthermore, we evaluate if these parameters correlate with the clinically suspected underlying pathophysiological process (inflammatory and ischemic NP-SLE), the clinical status before and after treatment or if they are related to different NP-SLE syndromes. Both DTI and 1H-MRS have been assessed in SLE patients. Although DTI is sensitive to changes in tissue microstructure, this technique lacks specificity in identifying the source of these changes. On the other hand, 1H-MRS does not provide structural information but provides cell-type specific information. In **Chapter 7** we combine both techniques using diffusion-weighted magnetic resonance spectroscopy (DW-MRS) performed at ultrahigh field (7-Tesla) to analyze the cell specific properties of tissue microstructure in the corpus callosum of SLE patients by probing the diffusion of intracellular brain metabolites.

Part III addresses the improvement of diagnosis and the outcomes in NP-SLE patients.

While in an acute clinical setting, recognizing the cause of the NP manifestations in a SLE patient can be difficult, at follow-up, the diagnosis may be assessed more reliably since the clinical course and response or failure to treatment provide diagnostic information. In **Chapter 8** we analysed the contribution of reassessment in the attribution process of NP manifestations to SLE or other aetiologies in a large, prospective and multidisciplinary assessed NP-SLE cohort. Moreover, our results were compared to all available attribution models for NP events occurring in SLE. The relationship between the different underlying pathophysiological process of these NP manifestations presenting in SLE and the clinical and patient's reported outcomes were assessed in **Chapter 9**.

Finally, **Chapter 10** includes a summary and general discussion on the findings that are described in this thesis. A summary of this thesis in Dutch is provided in **Chapter 11**.

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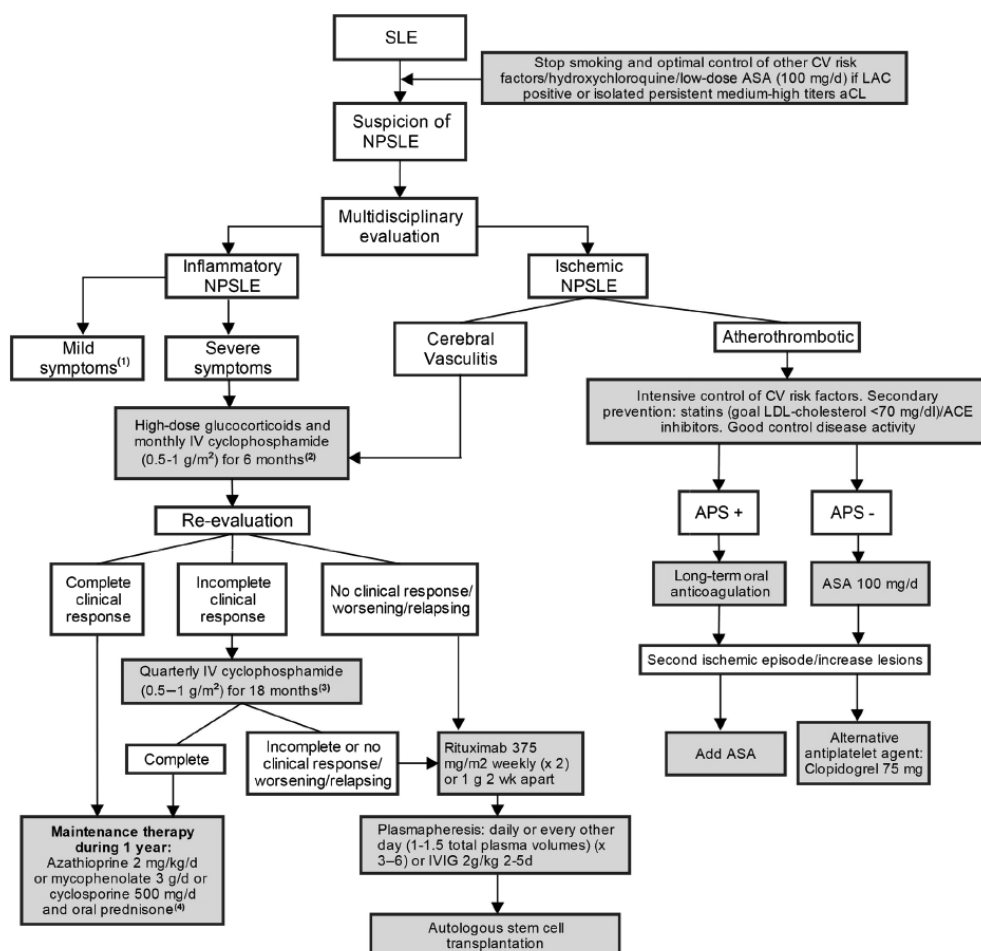
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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Suggested pathogenetic factors in neuropsychiatric systemic lupus erythematosus

Genetic
TREX1 gene
HLA-DRB1*04
STAT4 rs10181656
Autoantibodies
Brain cells and constituents: neuronal, brain reactive, gangliosides, neurofilament (alpha internexin), antibrain synaptosomal, anti-GFAP, Anti UCH-L1
Brain neurotransmitters: Anti-NMDA/NR2, GABA-B
aCL/ LAC/ β 2-Glycoprotein I
Sm/ Ro (SSA)/ U1 RNP/ Ribosomal proteins/ Histone
Endothelial cells
Other: MAP2, serum lymphocytotoxic antibodies, triosephosphate isomerase, Hsp70, alpha-tubulin, peroxiredoxin 4, splicing factor, SFRS3, Nedd5
Cytokines
Interleukines: IL-1, IL-2, IL-6, IL-8, IL-10, TNF, APRIL, IFN- α , INF- γ
Chemokines: CCL5, CCL2 (Monocyte chemotactic protein 1), CXCL10 (IP-10)
Accelerated atherosclerosis
Traditional risk factors
Inflammatory risk factors
SLE related risk factors (disease activity/duration, aCL and LAC, lupus nephritis, prednisone, low Vitamine D)
Other SLE specific factors
SLE disease activity
Heart valve disease
Immune complexes
Complement deposition
Others
PAI-1

aCL: anticardiolipin antibodies; APRIL: a proliferation-inducing ligand; CCL: Chemokine ligand; CNS: central nervous system; CSF: cerebrospinal fluid; CXCL: C-X-C motif chemokine; IL: interleukin; INF: interferon; GABA-B: Gamma-aminobutyric acid type B receptors; GFAP: Glial fibrillary acid protein; HLA: human leukocyte antigen; IP: interferon gamma-induced protein; LAC: lupus anticoagulant; MAP-2: Microtubule-associated protein 2; NMDA/NR2: N-methyl-D-aspartate receptor; PAI: Plasminogen activator inhibitor; RNP: ribonucleoprotein; SSA: Sjögren's syndrome-related antigen A; SLE: systemic lupus erythematosus; TNF: tumor necrosis factor; UCH-L1: Ubiquitin carboxyl-terminal Hydrolase isozyme L1



Supplementary Figure 1. Therapeutic approach for neuropsychiatric systemic lupus erythematosus (NP-SLE) based on available evidence and data from the Leiden NP-SLE-cohort. Combination of immunosuppressive therapy and secondary prevention may be used in the same patient when both ischemic and inflammatory pathogenic mechanisms are suspected. (a) In patients with mild symptoms or when non-specific NP-SLE syndromes (headache, anxiety, mood disorder, cognitive disorder) are suspected to be related with SLE symptomatic therapy may be sufficient, or glucocorticoids < 0.5 mg/kg/d +/- azathioprine 2 mg/kg and reevaluation of symptoms after 3-6 months may be considered. (*) In patients with severe NP-SLE, pulses of methylprednisolone 1 g/day for 3 days can be indicated; (**) Prednisone in a tapering dose; (***) Prednisone < 7.5 mg/day when possible. Azathioprine or other DMARDs such as mycophenolate depending on expertise or other concomitant organ SLE involvement. ASA= acetylsalicylic acid; APS = antiphospholipid syndrome; IVIG = intravenous immune globulin; LAC = lupus anticoagulant; aCL = anticardiolipin; NP-SLE = neuropsychiatric systemic lupus erythematosus; SLE = systemic lupus erythematosus.

part 1

LABORATORY

BIOMARKERS

2

C1Q DEFICIENCY AND NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

Magro-Checa C*, van Schaarenburg RA*, Bakker JA, Teng YK,
Bajema IM, Huizinga TW, Steup-Beekman GM, Trouw LA

** These authors contributed equally to this work.*

ABSTRACT

C1q deficiency is a rare immunodeficiency, which is strongly associated with the development of systemic lupus erythematosus (SLE). A mutation in one of the C1q genes can either lead to complete deficiency or to low C1q levels with C1q polypeptide in the form of low-molecular weight (LMW) C1q. Patients with C1q deficiency mainly present with cutaneous and renal involvement. Although less frequent, neuropsychiatric (NP) involvement has also been reported in 20% of the C1q-deficient patients. This involvement appears to be absent in other deficiencies of early components of the complement classical pathway (C1r/C1s, C2 or C4 deficiencies). We describe a new case with C1q deficiency with a homozygous G34R mutation in C1qC producing LMW-C1q presenting with a severe SLE flare with NP involvement. The serum of this patient contained very low levels of a LMW variant of C1q polypeptides. Cell lysates contained the three chains of C1q but no intact C1q was detected, consistent with the hypothesis of the existence of a LMW-C1q. Furthermore we provide a literature overview of NP-SLE in C1q deficiency and hypothesise about the potential role of C1q in the pathogenesis of NP involvement in these patients. The onset of NP-SLE in C1q deficient individuals is more severe when compared with complement competent NP-SLE patients. An important number of cases present with seizures and the most frequent findings in neuroimaging are changes in basal ganglia and cerebral vasculitis. A defective classical pathway, because of non-functional C1q, does not protect against NP involvement in SLE. The absence of C1q and subsequently some of its biological functions may be associated with more severe NP-SLE.

C1q-deficiency is a rare autosomal recessive inherited defect of the complement system caused by mutations occurring in one of the three C1q genes (C1qA; C1qB; C1qC).(1) Up to date, three different categories of mutations according to C1q level have been described. Apart from nonsense mutations and missense mutations leading to absence of C1q in serum, a missense mutation with detectable C1q levels has been described.(2) In the last case, some authors have demonstrated a low gradient density of C1q compared with healthy controls and is therefore called low molecular weight C1q (LMW-C1q).(3,4) Until now, a total of 77 C1q-deficiency patients in 49 families have been described.(5-7) An important variability in clinical presentation and outcome of these patients has been observed, ranging from asymptomatic patients to life-threatening encapsulated bacterial infections.(7-9) C1q-deficiency is also strongly related to systemic lupus erythematosus (SLE), being so far the most penetrant genetic factor predisposing to this disease. From all patients described, a total of 85% presented SLE-like symptoms while around 50% have been addressed as SLE according to the American College of Rheumatology diagnostic criteria.(1,3,4,7,8) Cutaneous involvement, oral ulcers and renal involvement are the most consistent manifestations. Although nervous system involvement is less frequent, with only 15 patients described, it can lead to severe neuropsychiatric (NP) symptoms.

Several reports, based on mouse models and/or in-vitro experiments describe that C1q plays a role in the brain during different developmental stages. C1q can be neuroprotective in the context of neurotoxicity induced by beta-amyloid,(10,11) but it is also reported to be involved in damage in the context of Alzheimer's disease.(12) It remains to be established to what extent C1q is involved in cognitive (dys)function in humans and how and in which stages of development C1q is protective or damaging to brain tissue.

In this report we describe a new C1q deficient patient with a G34R mutation in the C1qC chain leading to severe NP-SLE and review 15 SLE cases with C1q deficiency and NP involvement in the literature. Furthermore we analyse the biochemical structure of LMW-C1q in serum and in cell lysates.

PATIENT AND METHODS

Clinical presentation of the C1q deficient patient

A 24-year-old Dutch man was admitted to our hospital with a 2-day history of progressive weakness and sensory loss of the left arm, visual field loss on the left side and subjective cognitive complaints with regard to concentration and memory. He had been diagnosed with a SLE-like illness associated with C1q deficiency at the age of 10 months when he presented a butterfly rash and antinuclear antibodies (ANAs) positivity. The C1q deficiency was caused

by a homozygous g.5499G>A mutation at the C1qC gene, resulting in a G34R change in the C1q protein. Consanguinity was not reported.

At the age of three he developed polyarthritis, which was successfully treated with naproxen. At the age of seven he was admitted due to a relapsing polyarthritis and subacute cutaneous lupus, fever, aphthous ulcers, sunlight hypersensitivity, malaise and positive antibodies including ANAs, anti-Ro, anti-RNP70 and Sm. SLE was diagnosed and hydroxychloroquine 200 mg was started. Examination of the past medical history also included frequent upper airway and ear infections during the first 3 years of his life, Pertussis infection at the age of four, relapsing impetigo with a *Staphylococcus aureus* septicemia at the age of 19 years and relapsing virus varicella zoster infection after the age of 20.

On the current admission, the patient's body temperature was 37.7°C and blood pressure was 100/60 mmHg. Physical examination was remarkable with a butterfly rash (**Figure 1A**), severe sensory loss of the left arm, hyperesthesia of the left hand and homonymous hemianopsia of the left side. Laboratory tests revealed increased ESR (63 mm/h; normal <15) and CRP (13.7 mg/L; normal <5), a normal haemoglobin and complete blood count. Except for a reduced serum albumin level (31 g/L; normal 34-48), electrolytes, serum cholesterol, renal and liver testing were normal. Analysis of the urine was normal without casts or dysmorphic red cells. Protein excretion was 9.87 g/24h. The antibody profile was positive for ANAs, anti-Ro (>240 U/mL, normal <7), anti-RNP70 (79 U/mL, normal <5) and anti-Sm antibodies (>120 U/mL, normal <5). Anti-double-stranded DNA, anticardiolipin antibodies, Beta-2-GP1 antibodies, lupus anticoagulant, anti-phospholipase-A2-Receptor (PLA2R) and Anti-C1q autoantibodies were negative. At this time analysis of complement showed a classical pathway activity of 0% (normal > 74%), a low alternative pathway activity (22%, normal >39%), a low level of C1q (21 mg/L, normal 102–171 mg/L), whereas C3 (1.4 g/L, normal 0.9–2.0 g/L) and C4 (396 mg/L, normal 95–415 mg/L) were in the normal range. Blood and urine cultures were negative. Findings from the renal biopsy were compatible with a class V lupus nephritis, with a 'nearly full house' immunostaining showing a strong granular staining for IgG and a moderate granular staining for C3, both along the glomerular basement membrane; a slight granular staining for IgA and IgM, and kappa and lambda light chains, sometimes also in mesangial areas, but no staining for C1q (**Figure 1C**). Electron microscopy revealed subendothelial, subepithelial and mesangial deposits (**Figure 1D and E**). A low Minimental State Examination for the age and education of the patient (24, range 0-30) was found. A brain computed-tomography (CT) scan demonstrated a hyperdensity at the right frontal and parietal lobes and a contrast enhanced CT showed a bilateral filling defect in the transverse sigmoid sinus. A Magnetic Resonance Imaging (MRI) showed multifocal diffuse grey matter hyperintensities located in the fronto-temporal right lobe and high-intensity area on T2 in multiple regions of the right frontal and parietal lobes with high-

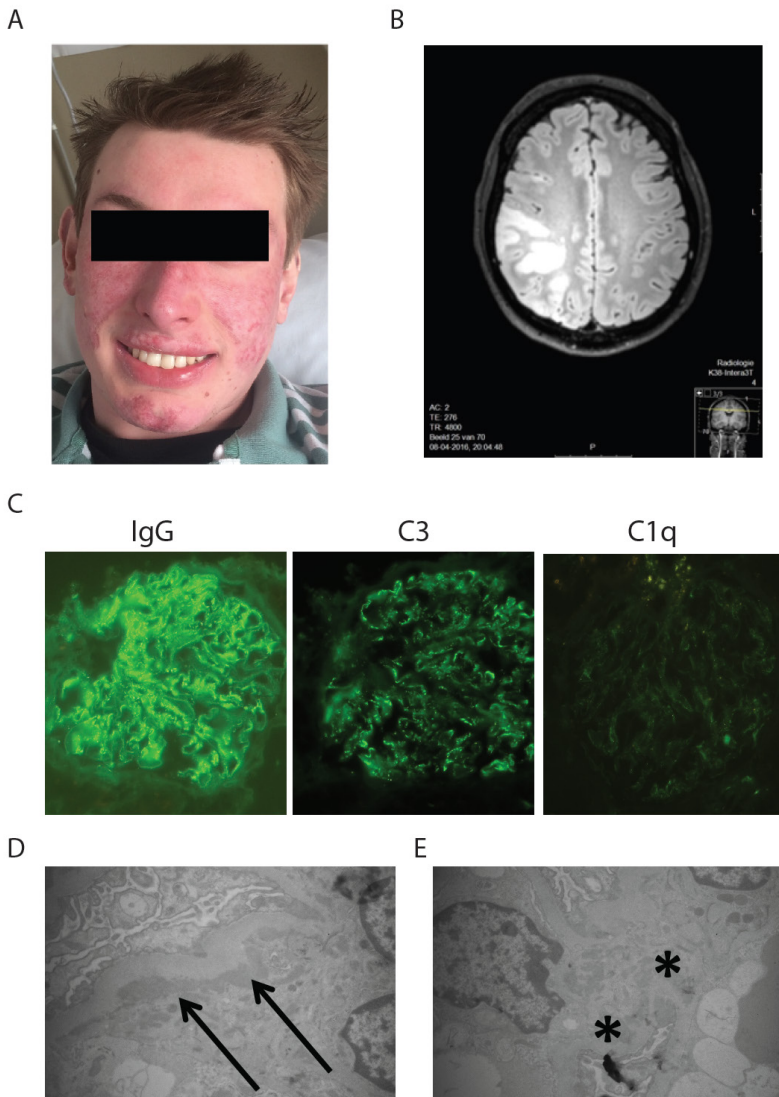


Figure 1. Clinical presentation of the C1q deficient patient. **A.** Malar rash and disoid lupus leading to mild scarring and atrophy **B.** 3-Tesla MRI brain (FLAIR image): multifocal diffuse grey matter hyperintensities located in the fronto-temporal right lobe and high-intensity area in multiple regions of the right frontal and parietal lobes **C.** Immunofluorescence staining of IgG deposition, C3 deposition and C1q deposition on the kidney **D.** Electron micrograph of the sub-endothelial deposition (arrows) of electron dense material. **E.** Electron micrograph of mesangial deposition (stars) of electron dense material.

intensities on the diffusion weighted imaging study (**Figure 1B**). A CT-angiography showed no signs of cerebral vasculitis. A diagnosis of lupus nephritis type V and NP-SLE with both inflammatory and ischemic phenotype were established. The patient was treated with daily clopidogrel 75 mg and intravenous methylprednisolone 1 gr 3 days plus oral prednisone

1 mg/kg/d in a tapering dose, and monthly intravenous cyclophosphamide 1 gm/m² for six months. Proteinuria improved dramatically in the first week and homonymous hemianopsia and cognitive dysfunction resolved after 2 weeks. After 3 months the patient still presented a mild sensory loss of the left arm. Both the patient and his parents provided informed consent for the studies.

Samples

Serum and PBMCs, isolated by Ficoll-Paque density gradient centrifugation were collected from the patient and an age matched control. During the admission a kidney biopsy was performed.

Microscopy

Slides for light microscopy evaluation were stained by hematoxylin and eosin, PAS and silver staining. Immunofluorescent stainings on cryostat sections were performed for IgA, IgG, IgM, C3, C1q and kappa and lambda light chains. Part of the renal specimen was used for electron microscopy. Pictures were taken with a JEM-1011 electron microscope (JEOL USA, Inc.) at various magnifications.

Gel filtration

Gel filtration experiments were carried out using the Äktaprime plus system (GE Healthcare, 11001313). 500 µl of filtered serum sample, either the healthy control serum or serum from the C1q deficient patient, was run through a Hiload Superdex Prep grade 200 16/600 column (GE Healthcare), using PBS as the running buffer. Fractions of 1ml were collected starting after half an hour for the duration of approximately 50 fractions. The protein levels in the fractions were analysed using a Pierce™ BCA Protein Assay Kit (ThermoFisher Scientific).

C1q ELISA

The levels of C1q in serum and supernatants were measured using an in-house developed ELISA. Maxisorp plates (Nunc) were coated with mouse anti-human C1q (Department of Nephrology, LUMC) in coating buffer (0.1 M Na₂CO₃, 0.1 M NaHCO₃, pH 9.6) overnight at 4°C. Plates were washed in PBS/0.05% Tween (PBS-T, Sigma). Then the wells were blocked with PBS/1% BSA for 1 hour at room temperature. After washing, the patient serum and control serum were added to the wells in a two-fold dilution series starting from 1:100 diluted in PBS/1% BSA/0.05% Tween (Sigma). After incubation for 1 hour at 37°C, the plates were incubated with rabbit anti-human C1q (DAKO) for 1 hour at 37°C and as detection antibody goat anti-rabbit HRP (DAKO) was used. Finally the substrate was added using ABTS (sigma). The C1q levels were measured at an absorbance level of 415 nm.

Western blot

Using western blot the composition of C1q was examined by detection of the three chains of the C1q protein. Due to the low amount of C1q present in the serum of the patient, we applied ten times more serum of the patient than the healthy donor. Cell lysates and supernatants of stimulated and unstimulated PBMCs of the healthy control and the patient were used in the same amount in reduced and non-reduced SDS conditions. The western blot was performed using previously described methods.(9)

Reconstitution complement activity assay

To exclude the possibility that next to C1q deficient the patients sample would also be deficient for C1r or C1s we performed assays to measure activation of the classical pathway of the patient serum by reconstitution of purified C1q. Plates coated with human IgG were incubated with 1% serum of the patient (diluted in GVB++; 0.1 % gelatin, 5 mM Veronal, 145 mM NaCl, 0.025 % NaN₃, 0.15 mM CaCl₂, 0.5 mM MgCl₂, pH 7.3) with or without addition of purified C1q (Quidel) in different concentrations. As a read-out C4 deposition was measured.

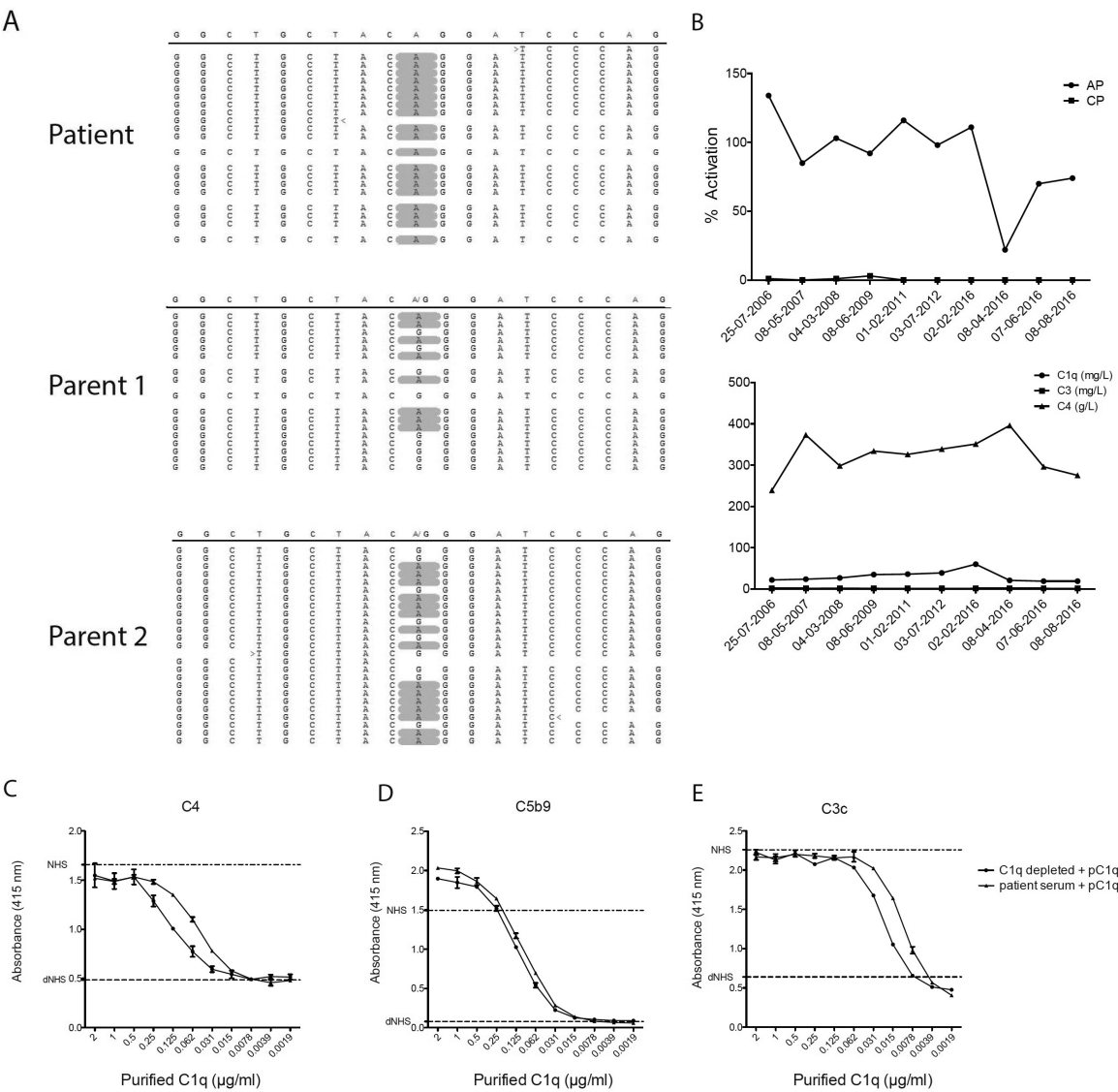
Sequencing

Genomic DNA was extracted from blood collected with tubes supplemented with EDTA. Sequencing of the complete C1q genes (C1qA, C1qB and C1qC), of both introns and exons was performed as before.(9) Deep-sequencing was performed using the 454 NGS Roche GS FLX Titanium platform. Data were compared to internal controls and to Human Genome build 19 as well as Human_v37_2 de dbSNP database v132 using the NextGENe software package for Next Generation Sequence Analysis (NGS) from Softgenetics. The effect of the mutation on splicing was in-silico analysed using the NetGene2 Server, <http://www.cbs.dtu.dk/services/NetGene2/>.

RESULTS

Detection of LMW-C1q in serum

With deep sequencing we identified a homozygous g.5499G>A mutation in the *C1qC* gene, resulting in a change in the C1qC chain where glycine was changed into an arginine at position 34 (G34A), while both parents show a heterozygous state of the mutation (**Figure 2A**). The routine diagnostics laboratory reported the patient to be completely lacking classical pathway activity (**Figure 2B**). This is compatible with a C1q deficiency, but to exclude that next to C1q also other factors would be deficient in the patient we performed a reconstitution assay where we add purified C1q to the serum of the patient and analyse C4 deposition. To compare the activity we performed the same analysis with C1q depleted serum. After adding purified C1q we were able to detect C4 deposition at a similar range as C1q depleted serum reconstituted with pC1q (**Figure 2C**). This indicated that the patient was able to produce C1r



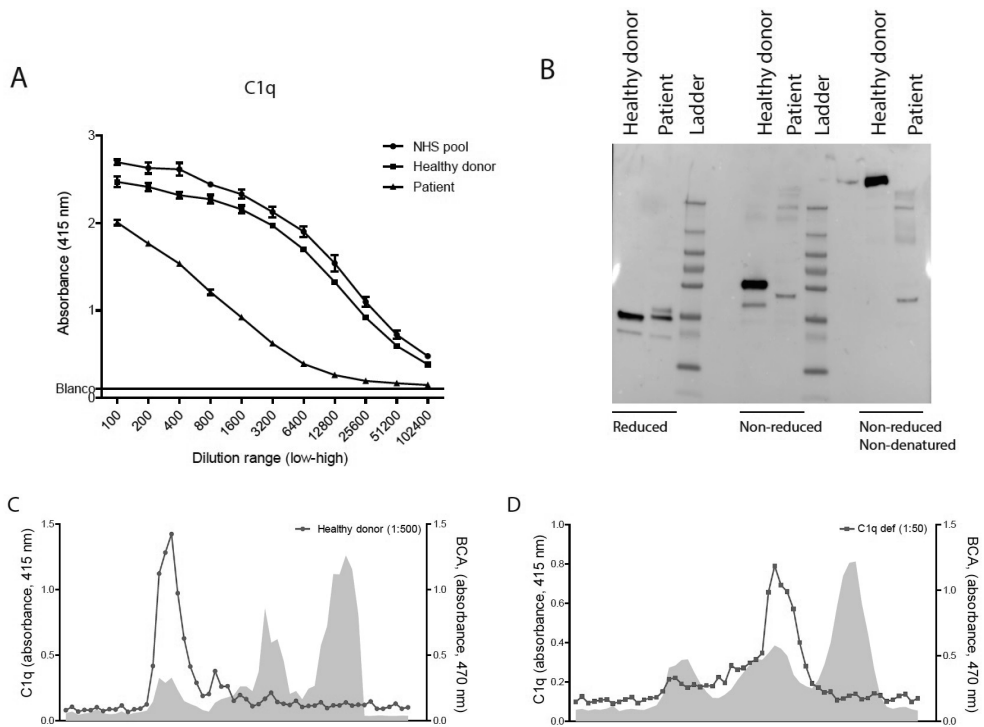


Figure 3. Detection of LMW-C1q in serum. **A.** C1q ELISA by using a dilution range of the serum of the C1q deficient patient (\blacktriangle), age-matched control (\blacksquare) and NHS (\bullet) as extra control. **B.** Western blot analysis of the serum in reduced, non-reduced and non-reduced/non-denatured conditions. As positive control an age-matched control is used. Patient serum was diluted 50x and the healthy control 500x. **C.** Protein analysis using a BCA protocol and C1q ELISA of different fractions after gel filtration of the serum of a healthy donor. **D.** Protein and C1q analysis of the patient.

and C1s, C2 and C4 and together with purified C1q was able to activate the classical pathway. Furthermore, we were also able to measure C5b9 and C3c deposition. This implied that there were no other complement deficiencies downstream in the complement system (**Figure 2D and E**). Using ELISA we could detect a decreased amount of C1q in the patient compared to the control samples (**Figure 3A**). We used western blot to examine the molecular structure of C1q in the patient serum. In reducing conditions all the three chains of the correct size are detected. However, using non-reducing conditions the dimers of C1q (2 x A-B and 1 x C-C) show an abnormal pattern. Using non-reducing/non-denaturing conditions we were able to detect high molecular weight C1q in the healthy control but not in the patient, suggesting that the C1q of the patient is of a LMW species (**Figure 3B**). With the usage of gel filtration the serum samples of the healthy donor and the patient were fractionated on size and with a BCA the amount of protein was analysed. While the protein profiles of both gel filtrations are similar, the location of C1q in the elution profiles is clearly different (**Figure 3C and D**).

Please note that since the serum of the patient was very low in C1q concentration we had to use different dilutions for the patient and the control in the ELISA to detect the presence of C1q in the fractions. These size-exclusion chromatography data confirm the LMW nature of C1q in the serum of the patient.

Composition of C1q in PBMC of the C1q deficient patient

To further examine the production of C1q by the cells of the patient by Western Blot, we stimulated PBMCs of the patient and the control with DXM and IFN- γ to upregulate the C1q production. Compared to the serum we loaded the same amount of lysate and supernatant to the lanes. In reducing conditions we see all the three C1q chains in the lysate of the PBMCs (**Figure 4A**). The dimers of C1q can also be detected in the lysates of the PBMCs from the patient. However, in non-reducing non-denaturing conditions, the dimers of C1q are detected, while additional bands are seen in the PBMCs of the patients, which may indicate the presence of intracellular LMW-C1q (**Figure 4B**). To examine the composition of secreted C1q, the supernatant of the PBMCs was analysed using western blot. The three chains of C1q were detected in the control supernatant as well as in the patient supernatant in reducing conditions. Surprisingly, the amount of C1q seems comparable between the patient and the control (**Figure 4C**). In non-reducing, non-denaturing conditions the high molecular size of C1q (460 kDa) is detected only in a very low concentration compared to the supernatant of the healthy control (**Figure 4D**).

C1q deficiency and NP-SLE

We performed an extensive electronic literature search from 1980 to 2016 using online databases (PubMed, Embase, Medline). We found 15 C1q-deficient patients with NP-SLE. All these patients presented at least one major central nervous system (CNS) manifestation.

Clinical and neuroimaging characteristics of these patients are summarized in **Supplementary Table 1**. Among all C1q-deficient patients with NP-SLE described so far in the literature, seizures was the most frequent NP symptom presented (10 patients; 67%). (6,13-20) Furthermore, five patients (33%) presented with a series of severe non-specific NP symptoms characterized by encephalopathy and difficulties to walk associated with cerebral infarcts and thought to be related with a cerebral vasculitis.(5,13,19-21) Transverse myelitis (6,22) and psychosis (14,22) were also present in 2 patients (13%). Neuroimaging of the brain showed as more frequent finding affection of basal ganglia (calcification or ischemic lesions) in 40 % of the cases (16,17,19-21,23) followed by cerebral vasculitis (27%) (13,15,20,21) and brain atrophy (20%).(6,17,24)

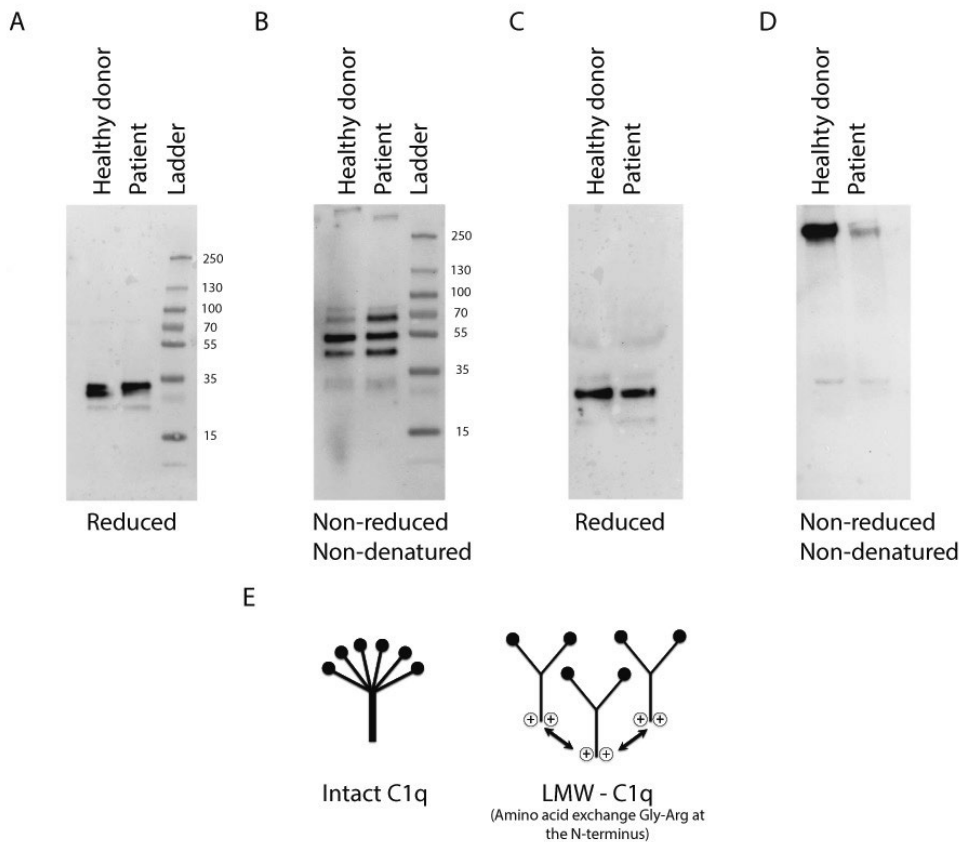


Figure 4. Analysis of stimulated cells from the C1q-deficient patient on the presence of C1q. **A.** Western blot analysis of cell lysates from stimulated PBMCs in reducing conditions, **B.** non-reducing and non-denaturing conditions. **C.** Western blot analysis of the supernatant of the PBMCs from the patient and the healthy donor (control) after 72h of culturing in reducing conditions. **D.** In non-reducing and non-denaturing conditions. The cell lysates and supernatant were added in the same amount. **E.** Schematic representation of intact C1q and LMW-C1q. In LMW-C1q positive charges are introduced in the collagen-like tail due the amino acid exchange Gly-Arg at the N-terminus.

DISCUSSION

The present study investigated an extremely rare case of C1q-deficiency due to non-functional LWM-C1q associated with a severe clinical phenotype presenting with membranous lupus nephritis and a mixed inflammatory and ischemic NP-SLE. C1q deficiency is a very strong susceptibility factor for the development of SLE where patients mainly present during childhood with skin or renal involvement and less frequently also with neuropsychiatric involvement.(7) Interestingly, although all the deficiencies of early components of the complement classical pathway are known to be a susceptibility factor for the development

of SLE-like disease, neuropsychiatric involvement appears to be absent in C1r/C1s, C2 or C4 deficiencies.(24,25) This makes us to speculate about the possible role of C1q in the underlying process leading to NP-SLE.

NP involvement in SLE-related C1q-deficiency presents with severe major CNS manifestations and its prevalence seems to be slightly higher than in complement competent NP-SLE patients (20% vs. <5%).(26) Seizures were the most common manifestation, presented in 60% of NP-SLE patients. In animal models, the production of C1q by neuronal cells was reported to lead to opsonisation of synapses in the developing postnatal CNS, which are next eliminated by microglia.(27) Several studies in murine models have described that C1q plays a role in the brain during different developmental stages. C1q can be neuroprotective in the context of for example beta-amyloid-induced neurotoxicity.(10,11) On the other hand, it is reported to be involved in damage in the context of Alzheimer's disease.(12) The complement system can hence facilitate normal neuronal development and protect against damage or contribute to neurodegenerative disease depending on yet to be identified triggers and timing. Currently it has not been formally studied whether C1q deficient patients have cognitive impairments. The neurological status of the current case completely normalised after the successful treatment of the SLE flare with immunosuppression, without any residual cognitive impairment. Moreover, studies using C1q knockout mice have demonstrated how a defective neocortical pruning of excessive excitatory synapses in these animals results in spontaneous and evoked epileptiform activity and increased intracortical excitatory connectivity.(28,29) This may explain the increased prevalence of seizures among these patients. Of note, neuroimaging demonstrated that a total of 40% of patients with C1q-deficiency presenting with NP-SLE showed involvement of the basal ganglia and in 27% of these patients findings were compatible with cerebral vasculitis. Neuroimaging changes in basal ganglia have been rarely reported in SLE patients. It has been suggested that these findings may represent vasogenic oedema and vascular changes occurring due to a vasculitic process localized in the basal ganglia probably due to immune-mediated underlying pathogenesis or effect of inflammation. Moreover, these MRI findings have been described to be reversible after starting immunosuppressive therapy.(30) SLE associated vasculitis may be associated with the deposition of immune complexes (ICs) in the endothelium. The deposition of these ICs may lead to endothelial cell activation and inflammatory cell infiltration.(31) Previous reports have proposed an important role of C1q in the clearance of apoptotic cells and circulating ICs.(32,33) Non-cleared debris due to absence of C1q may lead to helper T cells stimulation and autoantibody production.(34,35) Furthermore, in the last years C1q has been demonstrated to be of importance in vascular endothelial permeability and integrity. C1q and mannose binding lectin have been reported in in-vitro studies to help in the removal of atherogenic lipoproteins, which has been proposed as a link between C1q deficiency and

cardiovascular disease in SLE, as seen in our patient.(36,37)

Globally more than 60 patients are described with a C1q deficiency mostly due to a homozygous mutation. From these patients, 6 have the g.5499G>A mutation resulting in a G34A amino acid change and C1q deficiency.(4,14,16,17,20,38) Previous case reports that described the G34R mutation suggested the development of LMW-C1q, which is known as a non-functional C1q. In this study we demonstrate a C1q deficient patient with a low level of circulating C1q and an absence of classical pathway activity recorded over a long time period. Using sequencing we confirmed a homozygous G34R mutation. As suggested in previous studies, we also observed that the C1q present in this patient is LMW-C1q. Using western blot and gel filtration of the patient serum we detected a different molecular size of C1q in the patient serum at low concentrations. When we analysed the production of C1q by PBMCs we could detect all three C1q chains at a same concentration intracellularly, but after analysing C1q in the supernatant in non-reducing and non-denaturing conditions almost no fully folded C1q was detected. This confirms that the patient is able to produce all C1q chains but is unable to fold a complete functional C1q molecule. It is conceivable that the incorrectly folded C1q polypeptide chains have a strongly reduced half-life. Circulating C1q was completely absent after a flare of NP-SLE. This may suggest that there is consumption of the little C1q polypeptide that the patient produces. However, in the renal biopsy no C1q was detected, which could also indicate that it is not consumption of LMW C1q but rather a reduced production at the time of flare. Although temporary expression of LMW-C1q has been reported to occur during SLE flares or even in healthy persons, this production is temporary and involves only part of the total C1q pool.(39,40) In the current patient the production of LMW-C1q is genetically regulated and permanent and results in a completely defective classical pathway.

In conclusion, NP-SLE is a rare but severe complication in C1q-deficiency patients that must be diagnosed and treated promptly. The low level of LMW C1q observed in the patient did not allow any classical pathway activity, making the patient functionally C1q deficient. The role of C1q or its absence in the pathogenesis of NP-SLE merits further studies.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Cases reported with C1q deficiency and neuropsychiatric systemic lupus erythematosus

Age at onset/ Sex/ Flare	Country	Clinical features	NPSLE manifestation	Notes	Immunological tests	Complement functional tests	Neuroimaging	Medication	Mutation	Consequence/ Ref. Type C1q deficiency
1/M/ND	Yugoslavia	Malar rash, oral ulcers, photosensitivity, arthritis, LN (MPGN), NPSLE (Seizure)	Seizure	Recurrent infections Died at 13	ANA +, DNA+, SSA +, Sm+ RNP+	C1q=0	ND	Corticoids, frozen plasma, plasmapheresis and IVIG	g.8626C>T	Arg69X / Complete [17]
13/F/2	Saudi Arabia	Malar rash, discoid rash, oral ulcers, arthritis, leukopenia, thrombopenia, alopecia	Seizures, mononeuritis multiplex	ND	ANA+, DNA-, ENA- RNP+	C1q=0	ND	Corticoids, CYC	ND	ND [18]
7/F/7 and 20	Dutch	Malar rash, oral ulcers, LN, fever, alopecia, lymphadenopathy, myositis	Seizure, hemiplegia and lethargy. Probably cerebral vasculitis	Died at 20	ANA+, DNA-, RNP+ C1q<0.1 C1r/C1s=0 C3/C4: N CH ₅₀ <1% C1inh= N		Brain scintigraphy: multiple spots with activity mainly right sided, probably due to vasculitis	multiple Corticoids, CYC	ND	ND / Dysfunctional [13]
9/F/9	Japan	Malar rash, discoid rash, photosensitivity, oral ulcers, proteinuria (no biopsy), arthralgia	Seizure		ANA+, DNA-, SSA +, Sm+, RNP +	ND LMW C1q	Calcification of the basal ganglia and the temporal lobe (C1-scan)	Corticoids	g.5499G>A	Gly34AArg / Dysfunctional [16]
6/F/18,24 and 29	Germany	Malar rash, oral ulcers, photosensitivity, leukopenia, pleuritis, arthritis, glomerulonephritis (Type V) and Libman-Sacks endocarditis, peritonitis	Seizure and psychosis	Renal and heart failure died at 29	ANA+, DNA+, Sm+ C1q=28% C1r/C1s=N C2-C4f =N C3/C4= N CH ₅₀ =0 AP ₅₀ =N C1inh =N		ND	Corticoids, plasmapheresis, chlorambucil, CYC, cyclosporin, IVIG	g.5499G>A	Gly34AArg / Dysfunctional [14]
9/F/25	England	Malar rash, photosensitivity, leukopenia, alopecia	Seizure and cognitive dysfunction	Recurrent infections Died at 28	ANA +, DNA -, SSA +, Sm +, RNP + AP ₅₀ = N	C1q=0 CH ₅₀ <5% AP ₅₀ = N	Periventricular and basal ganglia calcification, with severe cerebral atrophy	Corticoids, Azathioprine, frozen plasma and plasmapheresis	g.8633delC	Gln71fsX137 / Complete [17]
5/F/ND	Saudi Arabian	Discoid lupus, photosensitivity, lupus nephritis (non-specified), alopecia	CNS involvement with cerebral atrophy, non-specified		ANA +, DNA -, SSA +, SSB +, Sm +	C1q=0 CH ₅₀ <5% AP ₅₀ = N	ND. Cerebral atrophy	Unknown	ND	ND / dysfunctional [24]
3/F/3 and 10	Inuit	Malar rash, discoid rash, photosensitivity, oral ulcers	CNS involvement, non-specified	Died at 10 of Pneumocystis +, RNP +, HF + carinii pneumonia	ANA +, DNA -, Sm +, RNP +, HF +	C1q< 6% C1s/C1r= N C3/C4= N CH ₅₀ = 1% AP ₅₀ =N C1inh= N MBL= N	ND	Corticoids, methotrexate,	g.13166G>A	Gly244Arg / Complete [23]
3/M/10	Pakistan	Malar rash, oral ulcers, fever	Cerebral vasculitis (Encephalopathy with global dysphasia, quadraparesis, generalized hypertonia and resting tremor)	Bacterial meningitis at 3	ANA +, DNA -, SSA +, Sm +	C1q=0 C2/C3/C4=N CH ₅₀ =0 AP ₅₀ =N	Bilateral infarction of his basal ganglia suggestive of a small vessel vasculitis	Corticoids, CYC, IVIG	ND	ND [21]

7/F/7	Arabian	Discoid rash, arthritis	Seizure, ACS, multiple ischemic lesions	Hyper IgM Syndrome Recurrent infections	ANA +, DNA +, SSA+, SSB+	C1q=5% CH ₅₀ =0	Multiple ischemic lesions involving both white matter and grey matter of hemisphere with left-sided predominance and also basal ganglia	Corticoids, CYC g. 5499G>A	Gly34Arg / Dysfunctional	[20]
6/F/6 and 15	Pakistan	Malar rash, oral ulcers, alopecia, Raynaud, fever and arthralgia	Seizure, cerebral vasculitis	Recurrent infections	ANA+, DNA-, Sm+	C1q=0 (ELISA) C3/C4= N CH ₅₀ =0 AP ₅₀ =N	Left frontal lobe infarct secondary to cerebral vasculitis	Corticoids, Azathioprine, frozen plasma	Gly55fsX83 / Complete	[15]
32/M/ND	Arabic	Thrombopenia, lymphopenia, AIHA, polymyositis	Seizure, transverse myelitis	Recurrent infections Died of bacterial septic shock and multi-organ failure	ANA+, RNP+, Ribosomal P +, ACA +	C1q= Normal MRI brain and spine: brain atrophy, thoracic spinal cord atrophy		Corticoids, CYC Codon 48 Bohain	Gly63Ser / dysfunctional	[6]
1/M/ND	Brasil	Photosensitivity, pericarditis	Psychosis and transverse myelitis	Cutaneous pyogenic infections and septic shock	ANA +, Sm +, RNP +	C1q=0 AntiC1q=0		ND	ND	[22]
1/F/2 and 4	Malese	Malar rash, oral ulcers, fever, Raynaud, vasculitic lesions fingers	Seizure, cerebral vasculopathy/vasculitis with several strokes, encephalopathy associated with spasticity	Salmonella infection	ANA +, DNA +, Ribosomal P +	C1q=0 CH ₅₀ =0	Bilateral frontal infarcts and basal ganglia calcification. Acquired myo-myoma pattern with bilateral occlusive disease of the terminal segments of the internal carotid arteries and associated basal collaterals. Perfusion studies marked hypoperfusion of the left hemisphere.	Corticoids, Azathioprine, MMF, CYC frozen plasma	Gly96Alafs / Complete	[19]
1/M/9	Iraq	Malar rash, LN (Type II), fever	CNS involvement, non-specified (Lethargy, difficulty to walk)	Recurrent infections Dead at 9. 4 months after allo-HSCT	ANA +, SSA +, RNP +	ND	MRI: Contrast enhancement in the left putamen in T1-weighted sequences. Enhanced signal in the right basal ganglia and capsula interna.	Rituximab, frozen ND plasma, exchange	Gin208X / Complete	[5]
1/M/24	Dutch	Malar rash, photosensitivity, oral ulcers, arthritis, LN (Type V)	Cognitive dysfunction, CVD	Recurrent infections	ANA +, DNA -, SSA+, Sm +, RNP +	C1q=low 20% MRI: Multifocal diffuse matter hyperintensities located in the fronto-temporal right lobe and high-intensity area on T2 in multiple regions of the right frontal and parietal lobes with high-intensities on the diffusion weighted imaging study CT-angiography: no signs of cerebral vasculitis.		Corticoids, CYC g. 5499G>A	Gly34Arg / Dysfunctional	Present case

ACS: acute confusional state; CYC: cyclophosphamide; IVIG: intravenous immunoglobulin therapy; LN: lupus nephritis; MMF: mycophenolate mofetil; ND: non described; NPSLE: neuropsychiatric systemic lupus erythematosus

3

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

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ABSTRACT

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

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

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

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

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

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

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

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

activity and damage) and laboratory characteristics.

PATIENTS AND METHODS

Patient selection and clinical evaluation

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

Laboratory assessment

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

until it was analyzed for the levels of anti-C1q and C1q CIC by enzyme-linked immunosorbent assay (ELISA). These laboratory determinations were performed at the Rheumatology Laboratory (LUMC, The Netherlands). Anti-C1q antibodies and C1q CIC in serum were measured by the QUANTA Lite™ Anti-C1q ELISA and with the usage of the QUANTA Lite® C1q CIC ELISA (Inova Diagnostics, San Diego, CA, USA), following the protocol from the manufacturer. The reference intervals were defined as < 20 units/ml for anti-C1q and as < 4.4 µg Eq/ml for C1q CIC. These classifications were also used to classify the healthy subjects. Another set of blood samples was tested for aPL, anti-dsDNA, anti-Sm, anti-RNP, anti-SSA/Ro52 and anti-SSB/La antibodies in the routine clinical laboratory at the LUMC. IgG anti-dsDNA antibodies were detected using the Crithidia Luciliae indirect immune fluorescence technique (Immunoconcepts, Sacramento, USA). IgG antibodies against SS-A/Ro-52, SS-B/La, Sm, RNP and IgG and IgM anti-cardiolipine and anti-β2-glycoprotein I antibodies were detected were determined using a Phadia® 250 EliA fluorescence enzyme immunoassay (FEIA) (Thermo Scientific, Freiburg, Germany). Lupus anticoagulans (LAC) was determined using STA-Rack en STA Evolution coagulation analysers (Stago, Parsippany, USA).

Statistical analysis

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

Table 1. Comparison clinical data SLE and NPSLE

	SLE n = 112	NPSLE Total n = 92	Focal * n = 63	Diffuse * n = 34
Age, mean ± SD years	44.01 ± 13.78	40 ± 13.68^a	43.23 ± 13.86	33.21 ± 10.19^{b,d}
Sex, no. female/male	99/13	82/10	55/8	32/2
Age at diagnosis SLE, mean ± SD years	35.4 ± 14.93	32.45 ± 14.8	35.01 ± 15.98	26.34 ± 10.05^{a,c}
SLE disease duration, mean ± SD years	8.61 ± 8.55	7.83 ± 8.31	8.23 ± 8.7	7.57 ± 8.08
SLEDAI-2K	4 [0 – 19]	6 [0 – 22]^b	6 [0 – 22]^b	9 [0 – 22]^{b,c}
ACR 1982 criteria for SLE †				
Malar Rash	54 (48.2)	34 (37)	21 (33.3)	15 (44.1)
Discoid rash	25 (22.3)	12 (13)	9 (14.3)	4 (11.8)
Photosensitivity	50 (44.6)	31 (33.7)	22 (34.9)	9 (26.5)
Oral ulcers	40 (35.7)	32 (34.8)	19 (30.2)	14 (41.2)
Arthritis	79 (70.5)	63 (68.5)	41 (65.1)	26 (76.5)
Serositis	30 (26.8)	30 (32.6)	22 (34.9)	11 (32.4)
Renal disorder	33 (29.5)	19 (20.7)	9 (14.3)	12 (35.3)^c
Neurologic disorder	8 (7.1)	25 (27.2)^a	14 (22.2)	12 (35.3)
Hematologic disorder	50 (44.6)	44 (47.8)	29 (46)	17 (50)
Immunologic disorder	78 (69.6)	71 (77.2)	49 (77.7)	26 (76.5)
Positive ANA	111 (99.1)	89 (96.7)	61 (96.8)	32 (94.1)
Autoantibodies and complement †				
aCL IgG	8 (7.1)	27 (29.3)^b	21 (33.3)^b	7 (20.6)^a
aCL IgM	6 (5.4)	8 (8.7)	6 (9.5)	3 (8.8)
LAC	19 (17)	43 (46.7)^b	35 (55.5)^b	12 (35.3)^{a,c}
Anti-β2GP1 IgG ††	6 (5.4)	17 (18.5)^a	13 (20.6)^a	5 (14.7)
Anti-β2GP1 IgM ††	2 (1.8)	5 (5.4)	5 (7.9)	1 (2.9)
Antinuclear antibody	75 (66)	78 (84.8)^a	53 (84.1)^a	29 (85.3)^a
Anti-dsDNA	23 (20.5)	33 (35.9)^a	22 (34.9)^a	14 (41.2)^a
ENA	66 (58.9)	48 (52.2)	32 (50.8)	20 (58.8)
Anti-SSA/Ro52	57 (50.9)	30 (32.6)^a	21 (33.3)^a	11 (32.4)^a
Anti-SSB/La	19 (17)	8 (8.7)	5 (7.9)	4 (11.8)
Anti-RNP	12 (10.7)	18 (19.6)	11 (17.5)	8 (23.5)
Anti-Sm	4 (3.6)	12 (13)^a	7 (11.1)	6 (17.6)^a
C1q low	7 (6.3)	13 (14.1)	7 (11.1)	8 (23.5)^a
C3 low	29 (25.9)	42 (45.7)^a	24 (38.1)	22 (64.7)^{b,c}
C4 low	27 (24.1)	30 (32.6)	14 (22.2)	18 (52.9)^{a,c}
CH50	25 (22.3)	37 (40.2)^a	19 (30.2)	19 (55.9)^{b,c}
AP50	16 (14.3)	27 (29.3)^a	14 (22.2)	16 (47.1)^{b,c}
Anti-C1q high	34 (30.3)	41 (44.6)^a	26 (41.3)	17 (50)^a
C1q CIC high	43 (38.4)	40 (43.5)	27 (42.9)	15 (44.1)
Antiphospholipid syndrome				
APS diagnosis	4 (3.6)	22 (23.9)^b	26 (41.3)^b	6 (17.6)^a
Arterial thrombosis ever	19 (17)	48 (52.2)^b	43 (68.3)^b	7 (20.6)^d
Vascular thrombosis ever	6 (5.4)	15 (16.3)^a	13 (20.6)^a	3 (8.8)

aCL: anticardiolipin antibodies; ACR: American College of Rheumatology; ANA: antinuclear antibody; LAC: Lupus anticoagulant; NPSLE: neuropsychiatric SLE; SLE: systemic lupus erythematosus; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000.

* 5 patients were included in both groups

† Number and percentage per group

†† Only available in 150 (69/81) patients

a. P < 0.05 when compared with SLE

b. P < 0.001 when compared with SLE

c. P < 0.05 when compared with focal SLE

d. P < 0.001 when compared with focal SLE

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

RESULTS

Demographic data and clinical characteristics

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

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

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

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

a. Possible > 1 NPSLE syndrome per patient

* Focal neuropsychiatric-SLE: Aseptic meningitis, cerebrovascular disease, demyelinating syndrome, headache, movement disorder, transverse myelitis, seizure disorder.

** Diffuse neuropsychiatric-SLE: psychosis, acute confusional state, anxiety disorder, cognitive disorder, mood disorder.

† Non-specific diffuse NPSLE syndromes: mood disorder, anxiety and mild cognitive dysfunction.

†† Non-specific focal NPSLE syndromes: headache.

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

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

CH50 and AP50 and NP-SLE

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

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

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

Complement activation and complement components as predictor of NP-SLE

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

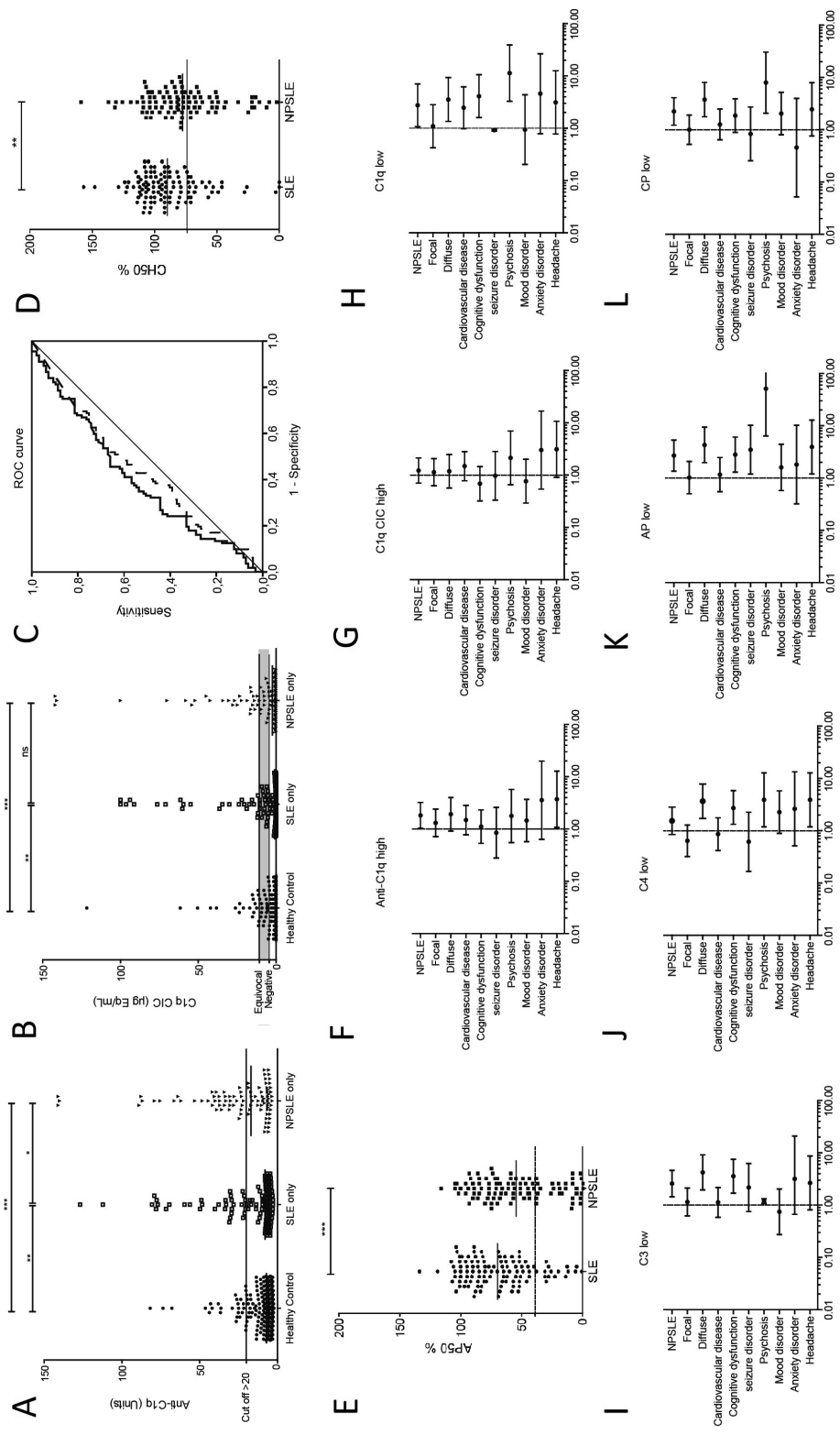


Figure 1. Serum titers of **(A)** anti-C1q antibodies and **(B)** C1q CIC in 92 consecutive patients with NPSLE, 112 patients with SLE and 200 HC. The titers of anti-C1q antibodies were significantly higher in patients with NPSLE than in the rest of SLE patients and HC ($P < 0.05$ and $P < 0.001$, respectively).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

DISCUSSION

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

We found an association between a low C4 and focal NP-SLE. Complement activation is known as an important mechanism of tissue injury in cerebral ischemia. Platelets bearing the complement activation product C4d are a known link between cerebrovascular inflammation and thrombosis. Moreover, they have been proposed as a specific biomarker for SLE diagnosis, and a relation with NP-SLE has also been suggested.(31,32) An increase in deposition of complement activation products, such as C4d, on platelets is associated with the presence of LAC, aCL and anti- $\beta 2\text{GP1}$ antibodies and it has been proposed as an essential mechanism in aPL-mediated thrombosis in SLE.(12,13,31,32) Serum hypocomplementaemia is commonly

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

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

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

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

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

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

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

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

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

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4

CLUSTER ANALYSIS OF AN ARRAY OF AUTOANTIBODIES IN NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

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Neuropsychiatric symptoms in patients with systemic lupus erythematosus (SLE) present a challenge to the clinician because they can be caused by the underlying disease (neuropsychiatric SLE; NP-SLE) or coexist independently.(1) No specific diagnostic test is available for NP-SLE. Reports on the associations between specific antinuclear autoantibodies and distinct NP-SLE syndromes have been conflicting (2,3,4,5), perhaps because of the laboratory tests used to detect these autoantibodies. New multiplex technologies for the detection of autoantibodies have emerged in the last years and might be helpful in diagnosing NP-SLE. We hypothesized that a cluster of autoantibodies could be associated with a specific NP-SLE syndrome or with focal or diffuse NP-SLE manifestations.

Therefore we used an addressable laser bead immunoassay test in patients who visited the NP-SLE clinic in Leiden, the Netherlands, a tertiary referral center for patients with SLE who have neuropsychiatric symptoms. Between September 2007 and February 2012, 133 patients with SLE who had neuropsychiatric symptoms were evaluated and diagnosed consecutively by a multidisciplinary team.(6) All patients fulfilled the revised SLE criteria of the American College of Rheumatology (ACR).(7)

In 81 (61%) patients a diagnosis of NP-SLE was established, whereas in the remaining patients the neuropsychiatric complaints were not attributed to SLE. The mean age of patients was 42.9 years (range 13–79), and 89% were female. The serum samples of all patients were analyzed using the FIDIS connective profile kit (Theradiag), a semiquantitative homogeneous fluorescent-based microparticles immunoassay for the simultaneous detection of these autoantibodies: anti-SSA (Ro60), anti-SSB, anti-TRIM21 (Ro52), anti-Sm, anti-Sm/RNP, anti-Jo1, anti-centromere B protein, antiribosomal-P, anti-dsDNA, anti-histone, anti-PmScl, and anti-PCNA. Further, anticardiolipin (aCL) IgG and IgM antibodies and lupus anticoagulant (LAC) status were available from the clinical evaluation. We performed hierarchical cluster analyses using R statistical software (version 3.0.2 for Windows) on (1) all autoantibodies from the microarray kit, and (2) all autoantibodies from the microarray kit plus the antiphospholipid antibodies (aPL), and we analyzed their associations with NP-SLE diagnosis, with the ACR NP-SLE syndromes, and with the groups of patients with either focal or diffuse NP-SLE manifestations (**Supplementary Figure 1**).(8) Statistical significance was defined as $p < 0.05$. In the first cluster analysis we identified 3 separate clusters of autoantibody profiles (no specific autoantibodies, anti-dsDNA/anti-SSA/anti-SSB/anti-TRIM21, and anti-Sm/RNP); however, no association with NP-SLE diagnosis or with NP-SLE syndromes was found. In the second cluster analysis, after inclusion of aPL, we identified 4 separate clusters of autoantibodies (**Table 1**). Three clusters identified in our analysis were similar to autoantibody profiles previously described in patients with SLE by To and Petri.(9) In our analysis we additionally identified a cluster characterized by the absence of specific autoantibodies. The frequency of major focal syndromes was significantly higher

in cluster 4 (anti-dsDNA/LAC/aCL IgM/IgG) than in other clusters ($p = 0.008$). Of the major focal syndromes, specifically cerebrovascular disease ($p = 0.030$) and seizure disorder ($p = 0.048$) were more frequent in cluster anti-SSA/anti-SSB/anti-TRIM21, and anti-Sm/RNP) were grouped and compared to cluster 4 (anti-dsDNA/LAC/aCL IgM/IgG), additionally an association was found for myelopathy ($p = 0.019$) in cluster 4. No association between an individual autoantibody and an NP-SLE manifestation was found, except for the following: aCL IgG with a NP-SLE diagnosis in general ($p = 0.019$), headache ($p = 0.004$), or psychosis ($p = 0.003$) and LAC with a seizure disorder ($p = 0.004$).

To our knowledge, this is the first report in NP-SLE that involves cluster analyses on autoantibodies retrieved by multiplex testing. In our present study we found an association between a cluster of autoantibodies (anti-dsDNA/LAC/aCL IgG/IgM) and NP-SLE. This association seems consistent with available literature.(3,4,5) This association was especially important in major focal syndromes and was stronger when patients with minor syndromes (headache, anxiety, cognitive dysfunction, and mild forms of depression) were excluded ($p = 0.001$). On the other hand, our study failed to show any associations between the other autoantibodies analyzed with the microarray kit or clusters of these autoantibodies and NP-SLE. The absence of more associations in our analyses hypothetically could also be due to the specific properties of this microarray kit, low numbers of patients per syndrome, or the fact that patients with NP-SLE as a group represent several pathogenic processes. Our data suggest that aPL are indispensable in the diagnostic investigations of NP-SLE in daily practice. Further studies concerning these and other autoantibodies are required. Possibly, to study the role of (clusters of) autoantibodies more appropriately in NP-SLE, their role in different pathogenic processes should be studied. Therefore our future work is aimed at finding associations between (clusters of) autoantibodies and advanced imaging results of the brain, as the best representative of tissue.

Table 1. Clustering of 133 patients with SLE and NP symptoms into four clusters by cluster analysis based on the results of a multiplex autoantibody profile and the presence of anticardiolipins

	Cluster 1 (None) (n = 23)	Cluster 2 (DNA/Ro/ La) (n = 40)	Cluster 3 (Sm/RNP) (n = 16)	Cluster 4 (DNA/LAC/ aCL) (n = 54)	P-value	P-value between individual and grouping clusters * 1-3 vs 4
Female, %.	86,9%	90%	93,7%	85,2%	0,782	0,290
Age, years, mean \pm SD years	43,4 \pm 15,3	44,8 \pm 15,5	32,5 \pm 10,6	42,7 \pm 15,6	0,056	0,251
No. of SLE ACR criteria met, mean \pm SD	4,3 \pm 0,8	4,8 \pm 1,1	4,8 \pm 1	4,7 \pm 1,2	0,350	0,837
Disease duration, mean \pm SD years	8,9 \pm 8,4	6,9 \pm 7,9	6,1 \pm 5,1	8,4 \pm 9,1	0,612	0,371
Duration of neuropsychiatric symptoms, mean \pm SD years	2,1 \pm 5,4	3,1 \pm 5,2	1,5 \pm 3,1	2,1 \pm 3,1	0,629	0,142
No. (%) of patients diagnosed as NPSLE	12 (14,8%)	19 (23,5%)	12 (14,8%)	38 (46,9%)	0,068	0,047
Total No. of CNS NPSLE ACR syndromes, mean \pm SD	1,2 \pm 1,4	1,1 \pm 1,5	1,2 \pm 1,2	1,8 \pm 1,7	0,101	0,331
Comparison of the number and frequencies of different CNS NPSLE ACR syndrome between each cluster**						
Cerebrovascular disease	5 (17,2%)	3 (10,3%)	3 (10,3%)	18 (62,1%)	0,03	0,009
Headache	4 (16%)	6 (28%)	2 (8%)	12 (48%)	0,853	0,287
Psychosis	2 (11,8%)	5 (29,4%)	0 (0%)	10 (58,8%)	0,251	0,092
Seizure disorder	1 (7,7%)	1 (7,7%)	1 (7,7%)	10 (76,9%)	0,048	0,007
Cognitive dysfunction	8 (19,5%)	8 (19,5%)	6 (14,6%)	19 (46,3%)	0,338	0,262
Myelopathy	0 (0%)	1 (14,3%)	0 (0%)	6 (85,7%)	0,096	0,019
Mood disorder	4 (20%)	7 (35%)	2 (10%)	7 (35%)	0,904	0,365
Anxiety disorder	3 (12,5%)	5 (20,8%)	3 (12,5%)	13 (54,23%)	0,228	0,097
Acute confusional state	1 (14,3%)	4 (57,1%)	1 (14,3%)	1 (14,3%)	0,370	0,138
Movement disorder	0 (0%)	1 (33,3%)	0 (0%)	2 (66,7%)	0,71	0,367
Comparison of the number and frequencies of focal and diffuse CNS NPSLE ACR syndrome between each cluster***						
Focal	7 (13,7%)	10 (19,6%)	6 (11,7%)	28 (54,9%)	0,06	0,010
Diffuse	9 (18,3%)	12 (24,5%)	6 (12,3%)	22 (44,9%)	0,341	0,341
Focal major syndromes†	5 (13,1%)	5 (13,1%)	4 (10,6%)	24 (63,2%)	0,008	0,001
Diffuse major syndromes††	5 (15,1%)	11 (33,3%)	3 (9,1%)	14 (42,5%)	0,920	0,539

Chi-square test. P values in bold face are statistically significant.

* No significant results were found for comparisons between other individual or grouping clusters.

** Values are the number (%) of patients. Demyelinating syndrome and aseptic meningitis did not occur.

***Values are the number (%) of patients with any focal or diffuse syndrome according with ACR nomenclature.

† Focal mayor syndromes include cardiovascular disease, chorea, seizures and myelopathy according with ACR nomenclature.

†† Diffuse mayor syndromes include acute confusional syndrome, mood disorder and psychosis according with ACR nomenclature.

ACR: American College of Rheumatology; CNS: central nervous system; NPSLE: neuropsychiatric systemic lupus erythematosus; SD: standard deviation; SLE: systemic lupus erythematosus; SLEDAI: systemic lupus erythematosus disease activity index.

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SUPPLEMENTARY MATERIAL

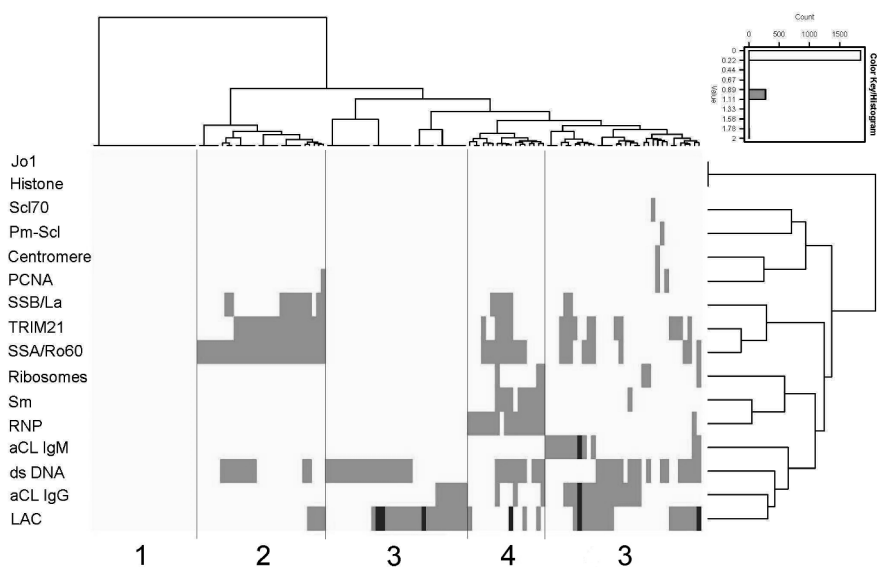


Figure 1. Heat map representation of hierarchical clustering of the autoantibodies analyzed with a microarray kit, anticardiolipins and lupus anticoagulant (columns) grouped by autoantibody-type (columns). Four separate clusters of autoantibody profiles (1. No specific autoantibodies; 2. DNA/Ro/La; 3 DNA/aCL/LAC; 4. Sm/RNP) were identified. Shades of red, orange and yellow represent the presence, absence and missing values of the antibody, respectively. aCL: anticardiolipin; LAC: lupus anticoagulant; PCNA: proliferating cell nuclear antigen; TRIM21: Tripartite motif-containing protein 21, also known as Ro(SS-A) (52 kDa).

part 2

NEUROIMAGING

BIOMARKERS

5

ARE SERUM AUTOANTIBODIES ASSOCIATED WITH BRAIN CHANGES IN SYSTEMIC LUPUS ERYTHEMATOSUS? – MRI DATA FROM THE LEIDEN NP-SLE COHORT

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ABSTRACT

Objective: The effect of serum autoantibodies on the brain of systemic lupus erythematosus (SLE) patients remains unclear. We investigated whether serum autoantibodies, individually and assessed in groups, are associated with specific brain-MRI abnormalities or whether these structural changes are associated with other SLE-related or traditional cardiovascular disease risk factors.

Methods: All patients underwent brain 3Tesla-MRI. White matter hyperintensities (WMHs), ischemic lesions, inflammatory-like lesions and cerebral atrophy were scored. Serum autoantibodies analyzed included lupus anticoagulant (LAC), anticardiolipine (aCL) IgG and IgM (first 3 also grouped into antiphospholipid autoantibodies (aPL)), anti-dsDNA, anti-SSA, anti-SSB, anti-RNP, and anti-Sm (the latter 5 grouped into SLE-related autoantibodies). Associations were assessed using logistic regression analysis adjusted for potential confounders. Furthermore, a sensitivity analysis including anti-Beta2 glycoprotein-1 antibodies (anti- β 2GP1) in the aPL group was performed and the potential modification role of the neuropsychiatric clinical status in the model was assessed.

Results: 325 patients (mean age 42 years (SD 14), 89% female) were included. The following MRI-brain abnormalities were found: WMHs (71%), lacunar infarcts (21%), gliosis (11%), micro-hemorrhages (5%), large hemorrhages (2%), inflammatory-like lesions (6%) and atrophy (14%). No associations were found between individual or total SLE-related autoantibodies and inflammatory-like lesions. A higher number of positive aPL was associated with lacunar infarcts (OR 1.37 (95%CI 1.02-1.99) and gliosis (OR 2.15 (1.37-3.37)). LAC was associated with lacunar infarcts in white matter (OR 3.38 (1.32-8.68)) and atrophy (OR 2.49 (1.01-6.15)), and aCL IgG with gliosis (OR 2.71 (1.05-7.02)). Among other variables, SLE patients with hypertension presented a higher chance for WMHs (OR 5.61 (2.52-12.48)) and lacunar infarcts in WM (OR 2.52 (1.10-5.74)) and basal ganglia (OR 8.34 (2.19-31.70)), while cumulative SLE-damage was correlated with lacunar infarcts in WM (OR 1.43 (1.07-1.90)), basal ganglia (OR 1.72 (1.18-2.51)) and cerebellum (OR 1.79 (1.33-2.41)). These associations were confirmed in the sensitivity analysis.

Conclusions: Brain abnormalities in SLE represent different underlying pathogenic mechanisms. aPL are associated with ischemic brain changes in SLE, while the presence of SLE-related serum autoantibodies is not related to inflammatory-like lesions. Hypertension and cumulative SLE-damage associate with ischemic MRI-brain changes in SLE, suggesting the importance of accelerated atherosclerosis in this process.

Nervous system involvement in systemic lupus erythematosus (SLE) leads to a heterogeneous group of neuropsychiatric (NP) manifestations. The two main underlying pathophysiologic processes in the brain resulting in NP-SLE are thought to be inflammation and ischemia.(1,2) The mechanisms that ultimately result in these pathophysiological changes and how they are related to each other remain poorly understood.

Over the past decade the type I interferon (IFN) was postulated to play a central role in SLE pathogenesis by promoting feedback loops progressively disrupting the peripheral immune tolerance and driving disease activity.(3) Recent discoveries implicate IFN-alpha together with the classical complement cascade as major pathways used by microglia for synaptic pruning in mice. Chronic peripheral inflammation in SLE may play a role in the aberrant activation of microglia and subsequently stimulate synapse loss, tagging inappropriate synaptic connections between neurons and subsequently leading to cerebral dysfunction.(4,5) The elevation of IFN-alpha activity has been related to autoantibody accumulation.(6) Moreover, several studies have described that autoantibody-containing immune-complexes may drive type I IFN activation.(7-9) Autoantibodies may also exert a direct effect upon neurons. The disruption of the blood brain barrier (BBB) integrity may permit the influx of neuropathic antibodies which may target synapses for engulfment by microglia.(10) Previous studies suggested that anti-dsDNA antibodies cross-react with N-methyl-D-aspartate (NMDA) receptors, and injecting these antibodies into mice causes hippocampal neuronal loss and cognitive impairment only when the BBB has been disrupted.(11,12)

Autoantibodies have also been associated with an ischemic pathogenic process. Antiphospholipid antibodies (aPL), especially lupus anticoagulant (LAC), have been related to intracranial thrombosis.(13) The complement cascade in close relation to aPL also plays a role in microvascular injury and NP-SLE pathogenesis.(14-16) Furthermore, accelerated atherosclerosis and traditional cardiovascular disease (CVD) risk factors have been involved in the ischemic process in SLE.(17)

Despite the fact that imaging abnormalities are not specific for NP-SLE, Magnetic Resonance Imaging (MRI) remains the neuroimaging technique of choice due to its superior soft tissue resolution. In a paired neuroimaging-autopsy study, Sibbit and coworkers observed that brain lesions in NP-SLE detected by MRI represent underlying cerebrovascular and parenchymal brain injury on histopathology.(18) Cerebral abnormalities that have been described in SLE on MRI are diverse; the most commonly reported is small vessel disease, especially white matter hyperintensities (WMHs) and lacunar infarcts, but also large vessel disease, inflammatory-like lesions (i.e. multifocal grey matter lesions) and brain atrophy are described.(19-23) While a fair number of studies on MRI abnormalities in SLE and NP-SLE have been published, only a few studies tried to unravel the mechanisms leading to these changes. It has been

proposed that focal lesions in SLE represent neuronal injury from various etiologies, ischemia and inflammation being the most important.(18) Luyendijk and coworkers described several distinct brain-MRI patterns in NP-SLE patients that were suggestive of different underlying pathogenic mechanisms.(24) The understanding of the pathogenic mechanisms leading to MRI abnormalities in SLE may be important to develop a rational prevention and treatment approach and in categorization of patients in further research.(23,24)

Based upon this knowledge, our primary hypothesis was that the total number of SLE-related autoantibodies is associated with inflammatory-like lesions and the number of aPL autoantibodies with ischemic changes as seen on brain-MRI. As a secondary objective we analyzed if MRI abnormalities were directly related to individual autoantibodies or otherwise with other SLE-related or CVD risk factors. Overall, we aim to investigate whether the underlying immune abnormalities in SLE are associated with pathophysiological changes as seen on brain-MRI.

METHODS

Study population

Between September 2007 and February 2016 a total of 325 SLE patients were seen in the Leiden NP-SLE-clinic and included in the present study. All patients fulfilled the ACR 1982 revised criteria for SLE.(25,26) Our hospital is a tertiary referral centre serving as a national referral centre for NP-SLE in the Netherlands. Patients are sent by a referral rheumatologist or other medical specialist to our center when SLE patients present NP manifestations. Therefore, all patients included in this study presented NP manifestations at time of the MRI. They were admitted for a 1-day period to the Leiden University Medical Center (LUMC). All patients underwent standardized multidisciplinary medical examination and extensive neuropsychological testing, serologic assessment and brain-MRI. Evaluations included in the multidisciplinary assessment have been reported in detail before.(27) The attribution process of NP-events to SLE and one of its underlying pathogenic mechanisms (ischemic or inflammatory) or to other etiologies was decided after multidisciplinary consensus and confirmed after re-assessment of patients at follow-up as described elsewhere.(28) This study was approved by the local medical ethics committee and all patients provided written informed consent.

MRI protocol and scoring

All subjects underwent a 3-Tesla MRI in the same scanner according to a standardized protocol (Achieva; Philips Healthcare). The scanning protocol included high-resolution T1-weighted, T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences, followed

by a T1-weighted sequence obtained after intravenous administration of gadolinium contrast agent. Scan parameters are shown in the **Supplementary Table 1**. All MRI examinations were visually examined by an experienced neuroradiologist (S. K.) who was blinded to clinical information. Areas of abnormalities were identified and their locations were documented. Deep WMHs were rated according to the visual Fazekas rating scale (ranging 0-3) on FLAIR images.(29) For analysis, we dichotomized this variable into low (Fazekas score <2) and high presence of WMHs (Fazekas score ≥ 2). The presence or absence of lacunar infarcts, large vessel infarcts, dural sinus thrombosis, cerebral micro-bleeds (CMBs), large hemorrhages, gliosis and inflammatory-like changes was also assessed. Lacunar infarcts were defined as ovoid areas of T2 hyperintense signal, with a hyperintense rim on FLAIR, measuring less than 20mm. These were distinguished from WMHs on the basis of central low signal on FLAIR. Lacunar infarcts were assessed in the white matter (WM), basal ganglia, thalamus, brainstem and cerebellum. Large vessel infarcts were defined as areas of T2/FLAIR hyperintensity involving the cortex and underlying WM confined to the distribution of a vascular territory. If these areas were restricted in diffusion, then the infarct was labeled acute. CMBs were defined as small (2-5mm), homogeneous and round areas of susceptibility artefacts on gradient echo images.(30) Gliosis was defined as a focal area of volume loss accompanied by T2 and FLAIR hyperintensity. If the area of gliosis was accompanied by hemosiderosis, it was deemed to have been the result of a previous hemorrhage. Presence of dural venous sinus thrombosis was suggested by the loss of normal flow voids in a dural venous sinus and absence of contrast enhancement within it and was confirmed by a MR Venogram showing loss of the corresponding normal flow signal. Lesions showing post-contrast enhancement following intravenous gadolinium injection, cortical hyperintensity and/or swelling on FLAIR or gyral restricted diffusion were thought to be inflammatory-type lesions according to Luyendijk *et al.*(24) Cerebral atrophy was assessed using the Pasquier scale, a four-point rating scale to assess cerebral atrophy ranging from 0 (absent) to 3 (severe cortical atrophy) and computed into low (Pasquier scale <2) and high (Pasquier scale ≥ 2). (31) See **Supplementary Figures 1-3** in additional supporting material.

Autoantibodies

Blood samples of all patients were collected from each participant at 08:00 a.m.. Determinations of serum anti-double-stranded DNA (anti-dsDNA), anti-Sm, anti-RNP, anti-SSA/Ro52, anti-SSB/La and aPL including anticardiolipin (aCL), anti-Beta2 glycoprotein 1 antibodies (anti- $\beta 2$ GP1) and LAC were performed the same day of the blood extraction in the routine clinical laboratory. IgG anti-dsDNA antibodies were detected using the Crithidia luciliae indirect immune fluorescence technique (Immuno Concepts, Sacramento, CA, USA). IgG antibodies against SS-A/Ro-52, SS-B/La, Sm, RNP and IgG and IgM aCL and anti- $\beta 2$ GP1 were determined using a Phadia 250 EliA fluorescence enzyme immunoassay

(FEIA) (Thermo Scientific, Freiburg, Germany). LAC was determined using STA-Rack and STA Evolution coagulation analyzers (Stago, Parsippany, NJ, USA).

Complement levels

Levels of C3 and C4 in serum were measured using laser nephelometry. Based on the normal limits for our laboratory, C3 <0.9g/l and C4 <95mg/l were defined as low.

SLE-related activity and damage

SLE disease activity was assessed with the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K).³² Permanent and irreversible damage due to SLE was calculated with the systemic lupus international collaborating clinics (SLICC)/American College of Rheumatology damage index (SDI).³³ All SLEDAI-2K and SDI values were calculated without NP variables. SDI was calculated without the diabetes variable.

Cardiovascular variables

At inclusion, data on age, gender, duration of SLE, medical history and CVD risk factors (smoking, hypertension, diabetes, dyslipidemia, body mass index (BMI)) were recorded, through interviewing the patient and by studying medical records. Furthermore, at this point glucose, triglycerides, total cholesterol, low-density (LDLc) and high-density lipoproteins cholesterol concentrations (HDLc) were determined. Hypertension was defined as elevated blood pressure >140/90mmHg or receiving antihypertensive therapy. Dyslipidemia was defined according to the National Cholesterol Education Program (Total cholesterol >5.2mmol/L, triglycerides >1.7mmol/L, LDLc >3.4mmol/L and HDLc <1mmol/L for men and <1.3mmol for women) or receiving dyslipidemia therapy.³⁴ Cigarette smoking was divided into current and ever smoking. Diabetes was defined as a fasting plasma glucose >7.0mmol/liter or receiving current anti-diabetic therapy. BMI was used as a continuous variable.

Statistical analysis

Demographic and clinical parameters were described as mean and standard deviations (SD) or proportions, as appropriate. The relationship between autoantibodies and MRI abnormalities was investigated through means of logistic regression analyses through which odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. First, an analysis of interaction between each of the autoantibodies (in groups and individually) and the diagnosis (NP-SLE vs SLE without NP-SLE) on the different outcomes was conducted. If interactions were statistically significant ($p < 0.1$), analyses were stratified in both subgroups. If differences in the relationships (between autoantibodies and MRI abnormalities) were considered clinically relevant, all analyses were further stratified for NP-SLE diagnosis. Subsequently, analyses were conducted with groups of autoantibodies including the number of positive aPL

(LAC, aCL IgG and IgM) ranging from 0-3 and the number of positive SLE-related antibodies (anti-dsDNA, anti-Sm, anti-RNP, anti-SSA/Ro52, anti-SSB/La) ranging from 0-5. Afterwards all these antibodies were included individually in separate models. Univariable regression was followed by multivariable regression. Variables from the univariable analysis with a $p < 0.20$ were included in the multivariable model. Some variables known from the literature as potential confounders were forced into the models to test whether they confounded the main relationships of interest. Significant variables or variables with a confounding effect on the relationship between autoantibodies and MRI abnormalities were kept in the final models. Because anti- β 2GP1 (IgG and IgM) were not tested in all patients, a sensitivity analysis was performed including these autoantibodies in the model as covariates first added in the aPL group (LAC, aCL IgG and IgM, anti- β 2GP1 IgG and IgM) ranging 0-5 and later analyzed individually. A $p < 0.05$ was used as level of significance. Statistical analysis was performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Three hundred twenty-five participants underwent brain-MRI scan. **Table 1** shows the clinical characteristics, autoantibody profile, CVD risk and SLE-related factors in the study population.

Brain-MRI abnormalities in SLE

Brain abnormalities in all SLE patients included in our cohort are shown in **Table 2**. WMHs were the most frequent radiologic finding with at least one WMH observed in 229 SLE patients (70.5%). Of all 325 patients, 61 (18.8%) had a Fazekas score of ≥ 2 . A total of 118 lacunar infarcts in 68 SLE patients were found. Cerebral atrophy (Pasquier ≥ 2) was found in 44 (13.6%) patients. A description of brain-MRI abnormalities according to the attribution of NP-events into the different clinical subgroups (non-NP-SLE, ischemic and inflammatory NP-SLE) is given in **Supplementary Table 2**.

Table 1. Clinical characteristics of the 325 SLE included patients

	n (%) or mean (SD)
Antibodies	
aCL IgG	63 (19.4%)
aCL IgM	31 (9.5%)
LAC	99 (30.5%)
Anti- β2GP1 IgG †	40 (14.4%)
Anti- β2GP1 IgM †	12 (4.3%)
ANA	315 (96.9%)
ENA	181 (55.7%)
Anti-ds-DNA	160 (49.2%)
Anti-SSA/Ro52	134 (41.2%)
Anti-SSB/La	43 (13.2%)
Anti-RNP	62 (19.1%)
Anti-Sm	41 (12.6%)
SLE-related factors	
SLEDAI-2K	5.1 (5.1)
SDI	1.2 (1.3)
C3 low	104 (32%)
C4 low	85 (26.2%)
Cardiovascular risk factors	
Hypertension	130 (40%)
Smoking	
Current smoker	86 (26.5%)
Ever smoker	160 (49.2%)
Never smoker	165 (50.8%)
BMI (kg/m²)	24 (21-28)
Dyslipidemia	186 (57.2%)
Diabetes mellitus	19 (5.8%)
Attribution of NP events	
Non-NP-SLE	204 (62.8%)
Ischemic NP-SLE	43 (13.2%)
Inflammatory NP-SLE	78 (24%)

aCL: anticardiolipin; β2GP1: Beta2 glycoprotein 1; BMI: body mass index; LAC: lupus anticoagulant; NP: neuropsychiatric; SD: standard deviation; SDI: systemic lupus international collaborating clinics (SLICC)/American College of Rheumatology damage index; SLE: systemic lupus erythematosus; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000.

† Only 278 patients were assessed for B2GP IgG and IgM

Relationship between groups of autoantibodies and SLE-associated MRI-brain lesions

The interaction between autoantibodies and NP-SLE status on MRI abnormalities was statistically significant in some of the cases (several not even statistically significant), but not clinically relevant (i.e. difference in the ORs between the 2 groups was not substantial); therefore, we decided to run the analysis in the whole population. Relationship between groups of autoantibodies and SLE-associated MRI-brain lesions are shown in **Table 3**. No relationship was found between the total number of SLE-related autoantibodies and MRI-brain abnormalities. Univariable analysis showed an association between an increasing

Table 2. Brain-MRI findings of the 325 SLE included patients

	n (%)
Normal MRI	83 (25.5)
Restricted diffusion	3 (0.9)
Gyral T2 hyperintensities	3 (0.9)
Gyral T1 hyperintensities	1 (0.3)
White matter lesions	
Periventricular WMHs	166 (51.1)
Deep WMHs	204 (62.8)
Subcortical	196 (60.3)
Fazekas score	
0	96 (29.5)
1	168 (51.7)
2	49 (15.1)
3	12 (3.7)
Basal Ganglia	6 (1.8)
Thalamus	5 (1.5)
Brainstem	25 (7.7)
Cerebellum	6 (1.8)
Lacunar infarcts	68 (20.9)
White matter supratentorial	38 (11.7)
Basal ganglia	24 (7.4)
Thalamus	10 (3.1)
Brainstem	7 (2.2)
Cerebellum	39 (12)
Large vessel infarcts	14 (4.3)
Sinus thrombosis	8 (2.5)
Focal white matter lesions	6 (1.8)
Parenchymal enhancement*	11 (3.4)
Leptomeningeal enhancement*	2 (0.6)
Inflammatory-like lesions*	19 (6)
Micro-haemorrhages	17 (5.2)
Large Haemorrhages	7 (2.2)
Gliosis	34 (10.5)
Cerebrocalcinosi	1 (0.3)
Cerebral atrophy (Pasquier scale)	
0	160 (49.2)
1	121 (37.2)
2	34 (10.5)
3	10 (3.1)

MRI: magnetic resonance imaging; SLE: systemic lupus erythematosus; WMHs: white matter hyperintensities.

* In 10 patients gadolinium was not used due to previous contrast allergy or because patient denied the use of contrast.

number of positive aPL antibodies and the presence of lacunar infarcts, CMBs, gliosis and atrophy. After adjustment for potential confounders, a significant relationship was only found for the increasing number of positive aPL antibodies and the presence of lacunar infarcts (OR 1.37 (1.02-1.99); $P < 0.05$) and gliosis (OR 2.15 (1.37-3.37); $P < 0.05$). None of the groups of autoantibodies was related to WMHs or inflammatory-like lesions. The clinical NP-status did not confound any of the relationships of interest and was therefore not kept in the models (data not shown). In the sensitivity analysis, after the inclusion of anti- $\beta 2$ GP1 in the aPL antibodies group, only the relationship between the total number of aPL and gliosis (OR 1.56 (1.10-2.20); $P < 0.001$) remained significant after multivariable analysis (**Supplementary Table 3**).

Relationship between individual autoantibodies, CVD risk factors, SLE-specific factors and SLE-associated MRI-brain abnormalities

Associations between brain-MRI abnormalities, individual autoantibodies, CVD risk factors and other SLE-related factors are shown in **Table 4**. Patients with hypertension had a higher odds of a high Fazekas score (OR 5.61 (2.52-12.48); $P < 0.001$). SLE patients with hypertension (OR 2.52 (1.10-5.74); $P < 0.05$), positivity for LAC (OR 3.38 (1.32-8.68); $P < 0.05$) and higher SDI (OR 1.43 (1.07-1.90); $P < 0.05$) presented a higher chance for lacunar infarcts in WM. Hypertension (OR 8.34 (2.19-31.70); $P < 0.05$), male gender and higher SDI (OR 1.72 (1.18-2.51); $P < 0.05$) were associated with lacunar infarcts in basal ganglia. Furthermore, SDI was also associated with the presence of infarcts in the cerebellum (OR 1.79 (1.33-2.41); $P < 0.05$). aCL IgG was associated with gliosis (OR 2.71 (1.05-7.02); $P < 0.05$) and LAC with cerebral atrophy (OR 2.49 (1.01-6.15); $P < 0.05$). Again, the clinical NP-status did not confound any of the relationships of interest and was therefore not kept in the models. All these significant relationships were confirmed in the sensitivity analysis. In the main analysis, anti-RNP antibodies were related to the presence of inflammatory-like lesions; however, this relationship was not confirmed in the sensitivity analysis. Other CVD risk factors were not associated with brain abnormalities (**Supplementary Table 4**).

Table 3. Relationship between groups of auto-antibodies and MRI-brain lesions in 325 SLE patients

	WMH		Ischemic changes [†]		Gliosis		Inflammatory-like changes		Atrophy [‡]	
	Fazekas [§]	OR (95% CI)	Lacunar infarcts	OR (95% CI)	Micro-hemorrhages	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Antiphospholipid antibodies (0-3)	Univariable	1.11 (0.79-1.54)	1.45 (1.07-1.97) ^a	OR (95% CI)	1.62 (1.01-2.63) ^a	OR (95% CI)	1.39 (0.84-2.30)	OR (95% CI)	1.51 (1.07-2.16) ^a	OR (95% CI)
	Multivariable*	1.18 (0.79-1.76)	1.37 (1.02-1.99) ^a	OR (95% CI)	1.46 (0.81-2.65)	OR (95% CI)	1.46 (0.82-2.61)	OR (95% CI)	1.395 (0.92-2.13)	OR (95% CI)
SLE related antibodies (0-5)	Univariable	0.81 (0.62-1.07)	0.82 (0.63-1.08)	OR (95% CI)	0.77 (0.48-1.23)	OR (95% CI)	0.76 (0.52-1.11)	OR (95% CI)	0.766 (0.55-1.07)	OR (95% CI)
	Multivariable*	0.84 (0.61-1.17)	0.91 (0.66-1.25)	OR (95% CI)	0.74 (0.42-1.30)	OR (95% CI)	0.88 (0.58-1.34)	OR (95% CI)	0.795 (0.55-1.16)	OR (95% CI)

MRI: magnetic resonance imaging; SLE: systemic lupus erythematosus; WMH: white matter hyperintensities.

Antiphospholipid antibodies included lupus anticoagulant, anticardiolipin IgM and IgG; SLE related antibodies included anti-dsDNA, anti-SSA/Ro52, anti-SSB/La, anti-Sm and anti-RNP.

*Multivariable analysis was adjusted for age, gender, hypertension, smoking (current or ever), BMI, dyslipidemia, diabetes mellitus, low C3, low C4, duration of SLE, SLEDAI-2K and SDI.

† Lacunar infarcts load includes: white matter, basal ganglia, thalamus, brainstem and cerebellum infarcts. No associations were found for large vessel infarcts, large hemorrhages and sinus thrombosis.

‡ Pasquier scale ≥ 2 .

§ Reference Fazekas score ≤ 1 .

a. $P < 0.05$; b. $P < 0.001$.

Table 4. Relationship between individual auto-antibodies and MRI-brain lesions in 325 SLE patients.

	WMH	Ischemic changes ^a				Inflammatory-like changes				Atrophy ^b		
		Lacunar infarcts		Cerebellum		Large vessel infarcts		Micro-hemorrhages			Gliosis	
	Fazekas ^c OR (95% CI)	White matter OR (95% CI)	Basal Ganglia OR (95% CI)	Thalamus OR (95% CI)	Cerebellum OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
aCL IgG	0.89 (0.31-2.61)	0.84 (0.32-2.52)	0.70 (0.15-3.14)	8.75 (1.24-62.09)^a	1.34 (0.50-3.61)	2.20 (0.50-9.82)	1.11 (0.31-3.95)	2.71 (1.05-7.02)^a	1.16 (0.34-4.00)			1.05 (0.40-2.72)
aCL IgM	0.54 (0.13-2.35)	0.38 (0.08-1.79)	1.02 (0.17-5.94)	5.46 (0.79-37.92)	0.94 (0.26-3.37)	5.31 (0.93-30.32)	0.61 (0.10-3.57)	2.01 (0.62-6.51)	1.72 (0.40-7.38)			0.91 (0.28-2.94)
LAC	1.64 (0.64-4.22)	3.38 (1.32-8.68)^a	0.68 (0.18-2.51)	1.24 (0.19-8.12)	1.87 (0.75-4.69)	1.04 (0.23-4.70)	2.03 (0.59-6.97)	1.98 (0.75-5.24)	1.62 (0.51-5.12)			2.49 (1.01-6.15)^a
Anti-dsDNA	0.77 (0.37-1.63)	0.62 (0.27-1.44)	1.29 (0.45-3.68)	0.42 (0.08-2.20)	1.31 (0.57-2.99)	0.34 (0.08-1.37)	0.95 (0.29-3.13)	1.08 (0.45-2.62)	1.09 (0.37-3.22)			0.50 (0.23-1.11)
Anti-SSA/Ro52	1.09 (0.47-2.55)	0.91 (0.37-2.23)	2.50 (0.76-8.25)	0.12 (0.01-1.76)	1.14 (0.46-2.97)	0.35 (0.06-1.99)	1.59 (0.49-5.13)	0.41 (0.13-1.36)	0.985 (0.33-2.98)			0.97 (0.41-2.30)
Anti-SSB/La	1.37 (0.43-4.37)	0.70 (0.16-3.07)	0.85 (0.17-4.09)	4.48 (0.36-55.37)	2.10 (0.59-7.47)	6.00 (0.85-42.26)	3.68 (0.79-29.30)	3.57 (0.80-15.91)	0.00			0.87 (0.25-3.09)
Anti-RNP	0.53 (0.17-1.64)	0.73 (0.22-2.46)	1.42 (0.35-5.71)	0.49 (0.05-5.19)	0.43 (0.11-1.62)	0.21 (0.02-2.09)	0.95 (0.20-4.43)	0.11 (0.01-0.91)^a	3.37 (1.01-10.56)^a			1.52 (0.56-4.14)
Anti-Sm	1.07 (0.25-4.51)	0.98 (0.23-4.14)	0.44 (0.04-4.57)	4.38 (0.29-65.31)	0.68 (0.13-3.61)	6.25 (0.82-47.80)	0.37 (0.04-3.62)	2.08 (0.46-9.38)	1.06 (0.24-4.70)			0.46 (0.09-2.28)
C3 low	0.91 (0.34-2.39)	0.92 (0.35-2.47)	0.96 (0.26-3.64)	3.66 (0.55-24.51)	1.44 (0.54-3.86)	1.01 (0.21-4.92)	3.93 (0.93-13.62)	1.07 (0.39-2.94)	0.45 (0.13-1.58)			1.75 (0.69-4.39)
C4 low	1.11 (0.43-2.86)	1.00 (0.35-2.84)	3.42 (0.90-13.06)	0.51 (0.07-3.70)	0.59 (0.20-1.75)	0.29 (0.04-2.15)	1.01 (0.27-3.80)	1.60 (0.57-4.55)	1.19 (0.34-4.23)			0.98 (0.37-2.61)
Age	1.05 (1.01-1.08)^a	1.02 (0.98-1.05)	1.01 (0.97-1.06)	1.03 (0.97-1.01)	1.03 (1.00-1.07)	1.02 (0.97-1.08)	1.06 (1.01-1.11)^a	1.01 (0.98-1.05)	1.00 (0.95-1.04)			1.05 (1.01-1.08)^a
Gender	0.59 (0.21-1.66)	1.57 (0.40-6.14)	0.24 (0.06-0.94)^a	2.25 (0.17-30.62)	0.30 (0.10-0.88)^a	0.27 (0.05-1.46)	1.08 (0.17-6.88)	0.65 (0.18-2.32)	2.67 (0.31-23.20)			0.63 (0.22-1.79)
Duration SLE	1.03 (0.99-1.07)	1.05 (1.01-1.10)^a	1.03 (0.97-1.08)	0.93 (0.84-1.03)	1.02 (0.97-1.06)	0.91 (0.82-1.01)	1.04 (0.98-1.10)	1.00 (0.95-1.05)	1.01 (0.94-1.08)			1.04 (1.01-1.08)^a
SLEDAI-2K	1.02 (0.93-1.11)	1.03 (0.94-1.12)	0.89 (0.77-1.03)	0.97 (0.81-1.17)	1.02 (0.94-1.11)	1.00 (0.87-1.15)	1.01 (0.90-1.14)	0.93 (0.84-1.03)	1.00 (0.91-1.11)			1.07 (0.99-1.16)
SDI	0.91 (0.68-1.22)	1.43 (1.07-1.90)^a	1.72 (1.18-2.51)^a	1.41 (0.73-2.71)	1.79 (1.33-2.41)^a	2.07 (1.23-3.50)^a	1.23 (0.83-1.82)	1.60 (1.17-2.18)^a	1.46 (0.97-2.18)			1.23 (0.97-1.62)
Hypertension	5.61 (2.52-12.48)^a	2.52 (1.10-5.74)^a	8.34 (2.19-31.70)^a	7.82 (1.06-57.77)^a	1.98 (0.89-4.44)	0.98 (0.25-3.88)	1.47 (0.47-4.57)	1.77 (0.72-4.36)	0.79 (0.25-2.55)			1.92 (0.91-4.07)
Smoking Current	2.45 (0.89-6.74)	1.24 (0.45-3.39)	1.47 (0.39-5.52)	1.45 (0.17-12.49)	0.59 (0.20-1.72)	0.46 (0.10-2.13)	5.12 (0.90-29.13)	0.84 (0.26-2.75)	0.41 (0.09-1.95)			1.06 (0.39-2.88)
Ever	0.52 (0.21-1.35)	2.22 (0.85-5.83)	2.21 (0.60-8.13)	0.69 (0.07-6.96)	1.64 (0.64-4.21)	4.78 (0.88-26.04)	0.25 (0.05-1.28)	1.06 (0.37-3.07)	1.37 (0.43-4.32)			1.04 (0.42-2.57)
BMI	0.99 (0.93-1.07)	0.99 (0.92-1.08)	1.02 (0.93-1.12)	1.07 (0.91-1.26)	1.01 (0.94-1.09)	1.04 (0.90-1.19)	0.98 (0.86-1.11)	1.01 (0.93-1.10)	0.89 (0.79-1.01)			1.05 (0.98-1.13)
Dyslipidemia	1.44 (0.71-2.93)	0.99 (0.45-2.20)	0.70 (0.25-1.92)	2.28 (0.45-11.69)	0.93 (0.42-2.08)	0.60 (0.16-2.22)	0.39 (0.12-1.26)	0.59 (0.25-1.42)	0.66 (0.21-2.10)			1.42 (0.67-3.01)
Diabetes	1.53 (0.37-6.29)	0.15 (0.01-1.62)	0.93 (0.13-6.68)	0.58 (0.03-10.67)	0.54 (0.10-2.90)	0.44 (0.02-9.30)	0.38 (0.03-5.87)	1.02 (0.17-6.20)	1.56 (0.15-16.67)			0.84 (0.21-3.46)

aCL: anticardiolipin; BMI: body mass index; C3: complement component 3; C4: complement component 4; LAC: lupus anticoagulant; MRI: magnetic resonance imaging; SLE: systemic lupus erythematosus; SLEDAI-2K: systemic lupus erythematosus disease activity index 2000; SDI: SLICC (systemic lupus international clinics) damage index; WMH: white matter hyperintensities.

† No association was found for lacunar infarcts in the brainstem, large hemorrhages and sinus thrombosis.

‡ Pasquier scale ≥ 2 .

* Reference Fazekas score ≤ 1 .

a. $P < 0.05$; b. $P < 0.001$.

DISCUSSION

In the present study, we have demonstrated the association of aPL, especially LAC, cumulative SLE-organ damage and several CVD risk factors and the presence of ischemic changes in the brain of SLE patients. On the other hand, SLE-related autoantibodies in serum, both total number and individual autoantibodies, were not associated with inflammatory-like lesions or other brain-MRI abnormalities.

Ischemic changes in the brain of SLE patients seem to be driven by aPL. Among all the serum autoantibodies analyzed, LAC, but not aCL or anti- β 2GP1 were associated with lacunar infarcts in the WM and also with cerebral atrophy. Previous reports have also found that LAC is a major risk factor for arterial thrombotic disease, especially for ischemic stroke in young women.(35,36) Furthermore, a higher prevalence of MRI abnormalities, mainly lacunar and large territorial infarctions, has been found in SLE patients with antiphospholipid syndrome;(21,37) LAC has been suggested to play the most important role in this association. (20) The relation between LAC and cerebral atrophy is more inconsistent. This relationship has been found using the Pasquier scale in a small study in SLE patients and another study including only NP-SLE patients without correction for other variables.(22,38) Other studies failed to demonstrate any significant association between LAC and cerebral atrophy, even when quantitative MRI-methods were used.(37,39) aCL IgG was related to gliosis. We hypothesize that gliosis seen in the brain of SLE patients is driven by aCL IgG and may be part of an underlying ischemic process or due to an autoimmune-mediated glial activation.

Inflammatory-like lesions in the brain of SLE patients were not found to be related to the total number of SLE-related autoantibodies. There may be different possible explanations for these negative findings. We report a low prevalence of inflammatory-like lesions (5.8%). In the presence of a low frequency of brain-MRI inflammatory-like lesions, it is difficult to capture factors associated with it due to a lack of power. Notwithstanding, it is difficult to interpret this frequency, as in the literature there are no other studies reporting the prevalence of inflammatory-like lesions. The MRI may have shown no abnormalities despite overt NP-SLE manifestations as it has been demonstrated with other quantitative MRI-techniques.(24,40) Moreover, the inflammatory-like lesions included are still a group of heterogeneous MRI changes which may reflect different pathophysiological changes. Another reason may be the autoantibodies selected for our analysis. In the future, other serum autoantibodies (i.e. anti-ribosomal P or anti-NMDA-receptor) and autoantibodies acquired from cerebrospinal fluid (CSF) may yield stronger associations when associated with quantitative MRI-techniques.

Other mechanisms, such as SLE-related and CVD risk factors, showed a correlation with brain-MRI abnormalities in SLE. Cumulative SLE organ-damage measured with SDI was

found to be associated with lacunar infarcts in WM, basal ganglia and cerebellum and with cerebral atrophy. Contrary to previous reports, SDI was not related to WMHs.(41) The presence of brain-MRI abnormalities in general has been previously related to higher disease severity scores and the need for more aggressive therapy.(42) Our data suggest that both infarcts and cerebral atrophy are related to chronic SLE-related damage in other organs. Therefore, patients with these brain-MRI abnormalities may have a more severe disease but also more damage due to systemic accelerated atherosclerosis affecting the whole arterial tree, which points to the importance of thrombotic/ischemic nature in leading to SLE-related damage.(43) In general population, the presence of atherosclerosis measured by carotid intima media thickness has been related to an increased risk for brain atrophy.(44) This accelerated atherogenesis in combination with other factors such as LAC and duration of disease may lead to brain infarcts and to increased brain atrophy in SLE. Early diagnosis, meticulous monitoring of SLE activity and effective use of immunosuppressive therapy may help avoiding SLE-related organ damage.

Among the CVD risk factors, hypertension was correlated with higher Fazekas score and lacunar infarcts. A relationship between long-standing hypertension and the presence of WMHs has been also described in a prospective study in a healthy population.(45) Furthermore, the strongest risk factor for ischemic stroke in general population is hypertension.(46) Wiseman *et al.* showed recently an association between Fazekas score and age and hypertension in SLE patients after unadjusted univariable association.(47) Our results confirm this association after correcting for multiple confounding factors and even when the influence of the clinical status (non-NP-SLE vs. NP-SLE) was taken into account. Contrary to a previous longitudinal SLE study(19), correlations between WMHs and aPL or SDI were not found. The different method used to assess WMHs in this previous study, semiautomatic volumetric measurements instead of Fazekas score, may explain these differences. Although hypertension and not antibodies such as aCL seem to play the most important role in the genesis of WMHs, we believe that there may be other unknown SLE and non-SLE-related contributing factors leading to WMHs. Compromised BBB integrity has been recently suggested as a contributor in the pathogenesis of WMHs in healthy population.(48) Future studies on SLE analyzing if adequate treatment of hypertension may prevent WMHs and atherosclerosis and the contribution of BBB permeability in the pathogenesis of WMHs are warranted.

Some limitations of this study should be taken into account. Due to the characteristics of our cohort and referral nuances, we were not able to measure ultrasound carotid atherosclerosis markers or to calculate the cumulative dose of corticosteroids and its effect in the brain could not be investigated. Lumbar puncture is not routinely performed in patients included in our cohort; therefore, we lack the results of CSF autoantibodies. Another limitation lies on

the fact that MRIs were scored only by one reader. The cross-sectional study design does not allow an interpretation of temporal or causal relationships. Another limitation may be the use of the visual Fazekas score and Pasquier scale for assessing WMHs and brain atrophy, respectively. Although these are widely used and accepted methods, the use of automated or semi-automated computer programs for quantifying lesion load and volume of WMHs and to assess measures of whole-brain atrophy may be more accurate.

Our study has important strengths. So far, most of the studies have focused in small groups of NP-SLE patients or in the difference between NP-SLE and SLE patients. In contrary, we decided to include all SLE patients in the study and focus in the nature of the MRI abnormalities. We have looked into the potential modification role of the NP clinical status in the association between the investigated antibodies and the brain-MRI alterations, which was not confirmed. Furthermore, since MRI abnormalities were probably used to establish a NP-SLE diagnosis, these studies would not avoid a certain level of selection bias and circular reasoning. It is also important to consider that a proportion of patients have abnormal MRI patterns without overt clinical symptoms or a normal MRI while presenting severe NP-SLE. (42) Thus, we are convinced that including all SLE patients in the study better captures the possible underlying pathophysiological mechanisms of these MRI abnormalities. Another strength of our study is that we assessed all patients with the same high field strength (3T) using a standardized MRI-protocol. To date, most studies included MRI-scans performed using different field strengths or using lower field strength (0.5-1.5T).

In summary, there is no indication that the total number or the individual SLE-related autoantibodies are associated with inflammatory-like lesions on the brain-MRI but the total number of aPL, especially the positivity for LAC, are associated with ischemic brain changes, mainly with lacunar infarcts and cerebral atrophy. Furthermore, cumulative SLE-organ damage and modifiable CVD risk factors, such as hypertension, contribute to these ischemic changes pointing out the importance of systemic accelerated atherosclerosis in SLE. We suggest that future studies should focus on CSF and other serum autoantibodies and their relationship with MRI abnormalities. Moreover, the inclusion of quantitative MRI-techniques at this point may help to better understand the underlying pathophysiological processes at a microstructural level.

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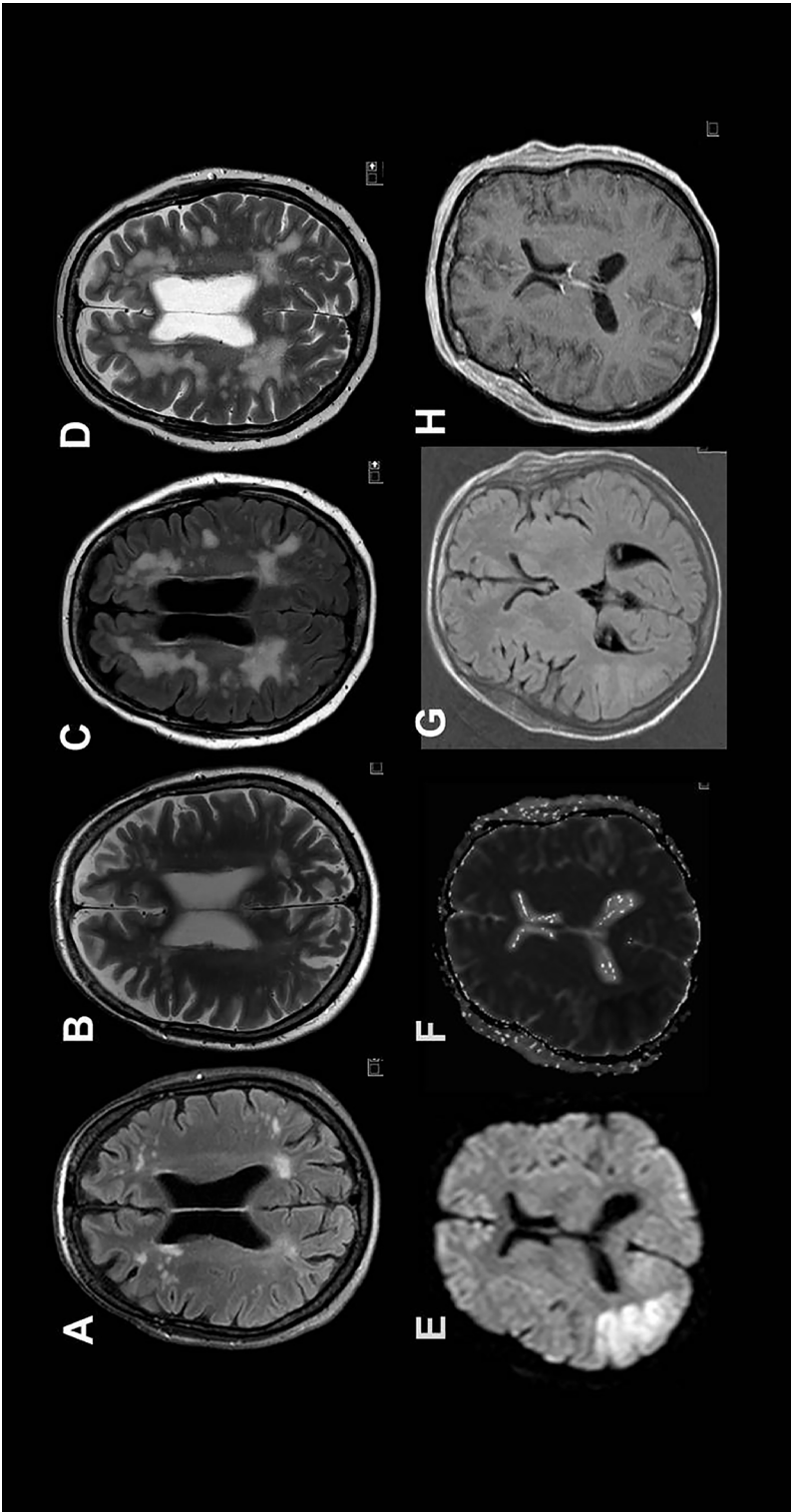
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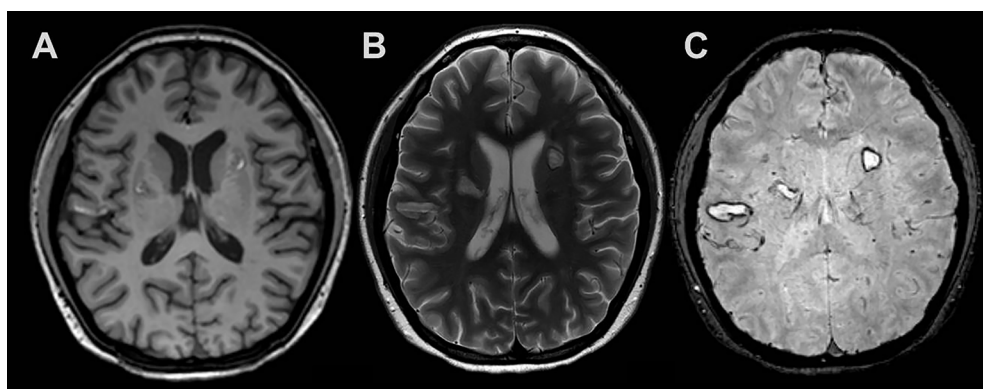
SUPPLEMENTARY MATERIAL

Supplementary Table 1. Scanning parameters

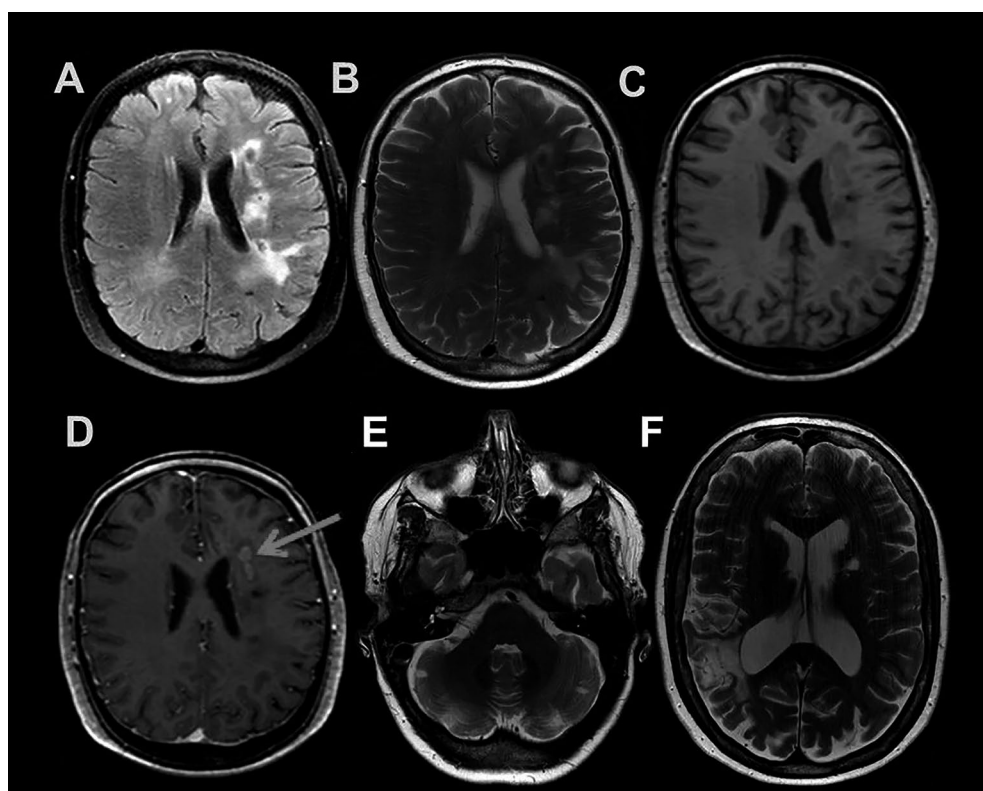
Sequence	T2-Weighted	3D T1-Weighted	3D FLAIR	3D T2-Weighted	3D T1-weighted with Gadolinium	SWI
Orientation	AX	AX	SAG	SAG	AX	AX
Scan Technique	Spin Echo	Gradient Echo	Inversion Recovery	Spin Echo	Gradient Echo	Gradient Echo
Scan Mode	Multi Slice	3D	3D	3D	3D	3D
TR	4158 ms	9.8 ms	4800 ms	2500 ms	9.8 ms	45 ms
TE	80 ms	4.6 ms	311 ms	317 ms	4.6 ms	31 ms
Inversion time (TI)			1650 ms			
Flip Angle	90	8		90	8	13
FOV	224 mm x 180 mm	224 mm x 177 mm	220 mm x 220 mm	250 mm x 250 mm	224 mm x 177 mm	250 mm x 181 mm
Acq. Matrix	448 x 319	192 x 152	200 x 197	208 x208	192 x 152	320 x 226
Resolution (acquired)	0.50 mm x 0.56 mm	1.17 mm x 1.17 mm	1.10 mm x 1.11 mm	1.20 mm x 1.20 mm	1.17 mm x 1.17 mm	0.78 mm x 0.78 mm
Resolution (reconstructed)	0.22 mm x 0.22 mm	0.88 mm x 0.88 mm	0.98 mm x 0.98 mm	1.12 mm x 1.12 mm	0.88 mm x 0.88 mm	0.78 mm x 0.78 mm
Slice Thickness	3.6 mm	1.2 mm	0.56 mm	0.6 mm	1.2 mm	0.8 mm
Number of Slices	40	120	310	300	120	180
Fold-over Direction	RL	RL	AP	AP	RL	RL
Band width	200 Hz	140.6 Hz	1041.7 Hz	387.7 Hz	140.6 Hz	111.1 Hz
SENSE Factor	RL = 2		AP = 2.6, RL =2	AP = 2, RL = 2		RL = 2
TSE Factor	16		182	100		
TFE Factor		154			154	
Fat Suppression			SPIR	SPIR		
NEX	2	1	2	1	1	1
Acquisition Time (min:sec)	02:54.6	04:37.6	04:04.8	02:32.5	04:37.6	03:17.0



Supplementary Figure 1. White matter hyperintensities. Fazekas 2, scattered hyperintensities in the periventricular and deep white matter beginning to confluence on axial FLAIR (A) and axial T2-weighted (B). Fazekas 3, confluent hyperintensities on FLAIR (C) and T2-weighted (D) images in the periventricular, deep white matter and subcortical white matter (no restricted diffusion). Acute infarct in right parieto-occipital lobe seen on diffusion weighted imaging (DWI) $b=1000$ (E), apparent diffusion coefficient (ADC) (F), axial FLAIR (G) and T1-weighted post-contrast (H) images. Restricted diffusion in the right parieto-occipital lobe i.e. high signal on DWI and corresponding low signal on ADC. Subtle high signal on FLAIR in the right parieto-occipital lobe and no change on the T1-weighted image.



Supplementary Figure 2. Subacute hemorrhages seen in bilateral lentiform nuclei and right parietal lobe, as bright signal on T1-weighted (A) and T2-weighted (B) images surrounded by a dark rim of hemosiderin on susceptibility weighted imaging (SWI) (C).



Supplementary Figure 3. Inflammatory lesion seen on axial FLAIR (A), axial T2-weighted (B), axial T1-weighted before contrast (C) and T1-weighted post-contrast (D). A new T2 and FLAIR hyperintense lesion in the left frontal periventricular white matter with enhancement (red arrow). Old infarcts in the cerebellar hemispheres (E), right parietal lobe and in the left basal ganglia (F) seen on axial T2-weighted images.

Supplementary Table 2. Brain-MRI findings in 325 SLE patients after attribution of NP events

	Non-NP-SLE (n = 204) n (%)	Ischemic NP-SLE (n = 43) n (%)	Inflammatory NP-SLE (n = 78) n (%)
Normal MRI	65 (32.9)	2 (4.7)	16 (20.5)
Restricted diffusion	0 (0)	0 (0)	3 (3.8)
Gyral T2 hyperintensities	2 (1)	0 (0)	1 (1.3)
Gyral T1 hyperintensities	0 (0)	1 (2.3)	0 (0)
White matter lesions			
Periventricular WMHs	95 (46.6)	30 (69.8)	41 (52.6)
Deep WMHs	115 (56.4)	32 (74.4)	57 (73.1)
Subcortical	113 (55.4)	31 (72.1)	52 (66.7)
Fazekas score			
0	74 (36.3)	6 (14)	16 (20.5)
1	99 (48.5)	22 (51.2)	47 (60.3)
2	24 (11.8)	12 (27.9)	13 (16.7)
3	7 (3.4)	3 (7)	2 (2.6)
Basal Ganglia	4 (2)	1 (2.3)	1 (1.3)
Thalamus	3 (1.5)	1 (2.3)	1 (1.3)
Brainstem	18 (8.8)	5 (11.6)	2 (2.6)
Cerebellum	2 (1%)	2 (4.7)	2 (2.6)
Lacunar infarcts	29 (14.2)	22 (51.2)	17 (21.8)
White matter supratentorial	16 (7.8)	12 (27.9)	10 (12.8)
Basal ganglia	11 (5.4)	7 (16.3)	6 (7.7)
Thalamus	3 (1.5)	5 (11.6)	2 (2.6)
Brainstem	3 (1.5)	3 (7)	1 (1.3)
Cerebellum	17 (8.3)	12 (27.9)	10 (12.8)
Large vessel infarcts	4 (2)	7 (16.3)	3 (3.8)
Sinus thrombosis	0 (0)	6 (14)	2 (2.6)
Focal white matter lesions	1 (0.5)	1 (2.3)	4 (5.1)
Parenchymal enhancement*	5 (2.5)	4 (9.3)	2 (2.6)
Leptomeningeal enhancement*	1 (0.5)	1 (2.3)	0 (0)
Inflammatory-like lesions*	6 (2.9)	7 (16.3)	6 (7.7)
Micro-haemorrhages	9 (4.4)	3 (7)	5 (6.41)
Large Haemorrhages	4 (2)	3 (7)	0 (0)
Gliosis	12 (5.9)	14 (32.6)	8 (10.3)
Cerebrocalcinosis	0 (0)	1 (2.3)	0 (0)
Cerebral atrophy (Pasquier scale)			
0	103 (50.5)	19 (44.2)	38 (48.7)
1	81 (39.7)	13 (30.2)	27 (34.6)
2	16 (7.8)	6 (14)	12 (15.4)
3	4 (2)	5 (11.6)	1 (1.3)

MRI: magnetic resonance imaging; SLE: systemic lupus erythematosus; WMHs: white matter hyperintensities.

* In 10 patients gadolinium was not used due to previous contrast allergy or because patient denied the use of contrast.

Supplementary Table 3. Sensitivity analysis including anti-β2 glycoprotein 1 IgG and IgM in the model. Relationship between groups of auto-antibodies and MRI-brain lesions in 278 SLE patients

		WMH		Ischemic changes [†]		Inflammatory-like changes		Atrophy [‡]	
		Fazekas [§]	OR (95% CI)	Lacunar infarcts	OR (95% CI)	Gliosis	OR (95% CI)	OR (95% CI)	OR (95% CI)
Antiphospholipid antibodies (0-5)	Univariable		1.03 (0.79-1.33)		1.18 (0.93-1.49)		1.11 (0.75-1.63)		1.43 (1.10-1.85)^a
	Multivariable*		1.04 (0.76-1.43)		1.07 (0.81-1.43)		1.15 (0.73-1.80)		1.36 (0.99-1.87)
SLE related antibodies (0-5)	Univariable		0.87 (0.65-1.17)		0.87 (0.65-1.16)		1.10 (0.72-1.70)		0.77 (0.53-1.11)
	Multivariable*		0.91 (0.64-1.29)		0.89 (0.64-1.24)		1.15 (0.72-1.85)		0.81 (0.53-1.22)

MRI: magnetic resonance imaging; SLE: systemic lupus erythematosus; WMH: white matter hyperintensities.

Antiphospholipid antibodies included lupus anticoagulant, anticardiolipin IgG, anti-β2 glycoprotein 1 IgG and IgM; SLE related antibodies included anti-dsDNA, anti-SSA/Ro52, anti-SSB/La, anti-Sm and anti-RNP.

*Multivariable analysis was adjusted for age, gender, hypertension, smoking (current or ever), BMI, dyslipidemia, diabetes mellitus, low C3, low C4, duration of SLE, SLEDAI-2K and SDI.

† Lacunar infarcts load includes: white matter, basal ganglia, thalamus, brainstem and cerebellum infarcts. No associations were found for large vessel infarcts, large hemorrhages, micro-hemorrhages and sinus thrombosis.

‡ Pasquier scale ≥ 2.

§ Reference Fazekas score ≤ 1.

a. P < 0.05; b. P < 0.001.

Supplementary Table 4. Sensitivity analysis including anti- $\beta 2$ glycoprotein 1 IgG and IgM in the model. Relationship between individual auto-antibodies and MRI-brain lesions in 278 SLE patients.

WMH	Ischemic changes ^a		Large vessel infarcts				Micro-hemorrhages		Gliosis		Inflammatory-like changes		Atrophy ^b	
	Fazekas ^c	Lacunar infarcts	White matter	Basal Ganglia	Cerebellum	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	
aCL IgG	0.84 (0.24-3.01)	2.21 (0.57-8.54)	0.96 (0.13-7.12)	1.24 (0.33-4.71)	4.55 (0.46-44.81)	1.34 (0.19-9.71)	4.90 (1.30-18.55)^a	1.97 (0.43-9.10)	0.82 (0.23-2.98)					
aCL IgM	0.54 (0.11-2.75)	0.16 (0.01-1.99)	1.12 (0.12-10.10)	1.43 (0.29-7.20)	2.35 (0.10-54.93)	0.12 (0.01-3.47)	5.07 (0.95-27.14)	1.25 (0.11-13.68)	0.32 (0.06-1.76)					
LAC	1.61 (0.61-4.25)	3.88 (1.33-11.35)^a	0.79 (0.19-3.38)	1.62 (0.56-4.73)	2.19 (0.32-15.01)	1.72 (0.35-8.50)	2.40 (0.74-7.81)	1.43 (0.38-5.29)	3.05 (1.01-9.21)^a					
β2-GP1 IgG	1.12 (0.29-4.40)	0.24 (0.046-1.26)	0.34 (0.03-3.72)	1.51 (0.37-6.15)	0.06 (0.01-1.31)	0.41 (0.04-4.37)	0.42 (0.09-1.83)	0.00	2.92 (0.73-11.74)					
β2-GP1 IgM	1.01 (0.14-7.06)	0.00	0.65 (0.04-11.43)	0.45 (0.05-3.81)	0.00	7.78 (0.34-177.26)	0.07 (0.01-1.15)	16.29 (0.43-615.05)	0.60 (0.07-5.39)					
Anti-dsDNA	0.78 (0.37-1.64)	0.48 (0.18-1.29)	1.14 (0.36-3.68)	1.17 (0.47-2.89)	0.74 (0.14-3.93)	0.51 (0.11-2.36)	1.85 (0.62-5.53)	1.475 (0.44-5.01)	0.57 (0.23-1.42)					
Anti-SSA/Ro52	1.10 (0.47-2.55)	0.97 (0.35-2.70)	3.00 (0.83-10.81)	1.18 (0.42-3.30)	0.62 (0.08-4.61)	1.14 (0.29-4.41)	0.53 (0.14-2.02)	1.56 (0.44-5.56)	1.23 (0.46-3.28)					
Anti-SSB/La	1.37 (0.43-4.37)	1.07 (0.23-4.99)	0.78 (0.14-4.01)	2.07 (0.54-7.93)	4.06 (0.38-43.50)	0.00	2.32 (0.38-14.28)	0.00	0.69 (0.15-3.11)					
Anti-RNP	0.53 (0.17-1.68)	0.63 (0.17-2.37)	1.27 (0.30-5.38)	0.42 (0.11-1.64)	0.24 (0.02-3.24)	1.32 (0.24-7.15)	0.13 (0.01-1.29)	3.01 (0.82-11.03)	1.93 (0.62-6.01)					
Anti-Sm	1.07 (0.25-4.51)	0.67 (0.12-3.78)	0.39 (0.04-4.19)	0.74 (0.13-4.19)	5.80 (0.37-90.33)	0.50 (0.03-7.42)	0.91 (0.08-9.84)	1.01 (0.17-6.03)	0.29 (0.03-2.57)					
Low serum C3	0.89 (0.34-2.39)	1.92 (0.62-5.94)	1.44 (0.34-6.20)	1.72 (0.60-4.95)	3.56 (0.41-30.69)	4.16 (0.88-19.71)	1.59 (0.48-5.29)	0.32 (0.07-1.476)	1.10 (0.37-3.25)					
Low serum C4	1.11 (0.43-2.86)	0.96 (0.30-3.10)	3.14 (0.77-12.81)	0.56 (0.18-1.75)	0.18 (0.01-2.52)	1.39 (0.32-6.10)	2.18 (0.64-7.40)	1.57 (0.37-6.74)	1.12 (0.37-3.40)					
Age	1.05 (1.01-1.08)^a	1.02 (0.97-1.06)	1.00 (0.95-1.06)	1.03 (0.99-1.07)	1.04 (0.96-1.12)	1.08 (1.01-1.14)^a	1.01 (0.96-1.06)	0.99 (0.95-1.05)	1.06 (1.02-1.10)^a					
Gender	0.60 (0.21-1.70)	0.93 (0.22-3.91)	0.20 (0.04-0.99)^a	0.37 (0.12-1.18)	0.15 (0.02-0.99)^a	1.07 (0.11-10.87)	0.64 (0.15-2.80)	2.30 (0.23-23.01)	0.63 (0.20-1.97)					
Duration SLE	1.03 (0.99-1.07)	1.04 (0.99-1.09)	1.02 (0.96-1.09)	1.01 (0.96-1.06)	0.94 (0.85-1.04)	1.04 (0.97-1.12)	1.01 (0.95-1.07)	1.01 (0.94-1.08)	1.06 (1.01-1.11)^a					
SLEDAI-2K	1.02 (0.93-1.11)	1.00 (0.91-1.11)	0.89 (0.76-1.03)	1.01 (0.93-1.11)	1.02 (0.86-1.20)	1.00 (0.86-1.16)	0.88 (0.78-1.01)	0.98 (0.88-1.10)	1.10 (0.99-1.21)					
SDI	0.91 (0.67-1.22)	1.60 (1.14-2.26)^a	1.87 (1.23-2.84)^a	1.85 (1.33-2.58)^b	1.49 (0.83-2.70)	1.20 (0.74-1.95)	1.39 (0.95-2.03)	1.52 (0.99-2.35)	1.45 (1.06-1.99)^a					
Hypertension	5.61 (2.52-12.49)^b	3.27 (1.21-8.82)^a	6.42 (1.52-25.52)^a	1.44 (0.59-3.50)	2.10 (0.38-11.61)	1.93 (0.44-8.46)	3.45 (1.08-11.03)	0.73 (0.20-2.64)	1.24 (0.52-2.94)					
Smoking Current	2.45 (0.89-6.76)	1.79 (0.58-5.49)	1.27 (0.31-5.22)	0.58 (0.19-1.80)	0.54 (0.09-3.29)	6.44 (0.90-46.17)	0.63 (0.16-2.37)	0.33 (0.05-2.17)	1.71 (0.57-5.12)					
Ever	0.52 (0.20-1.34)	2.82 (0.94-8.42)	2.02 (0.52-7.76)	1.39 (0.52-3.74)	5.10 (0.69-37.76)	0.26 (0.04-1.48)	2.07 (0.64-6.75)	1.11 (0.32-3.85)	0.99 (0.35-2.85)					
BMI	0.99 (0.93-1.07)	1.00 (0.92-1.10)	1.05 (0.95-1.15)	1.03 (0.95-1.11)	1.05 (0.90-1.22)	1.00 (0.86-1.17)	0.99 (0.91-1.10)	0.86 (0.74-1.01)	1.07 (0.99-1.15)					
Dyslipidemia	1.45 (0.71-2.95)	0.58 (0.23-1.46)	0.68 (0.23-2.00)	0.88 (0.37-2.09)	0.84 (0.18-3.81)	0.31 (0.05-1.20)	0.53 (0.19-1.50)	0.62 (0.18-2.13)	1.77 (0.74-4.21)					
Diabetes	1.53 (0.37-6.34)	0.18 (0.02-2.08)	1.39 (0.19-10.25)	0.54 (0.09-3.37)	1.47 (0.08-25.51)	0.00	2.33 (0.29-18.54)	1.47 (0.11-19.71)	0.45 (0.09-2.34)					

aCL: anticardiolipin; $\beta 2$ -GP1: Beta2 glycoprotein 1; BMI: body mass index; C3: complement component 3; C4: complement component 4; LAC: lupus anticoagulant; MRI: magnetic resonance imaging; SLE: systemic lupus erythematosus; SLEDAI-2K: systemic lupus erythematosus disease activity index 2000; SDI: SLICC (systemic lupus international clinics) damage index; WMH: white matter hyperintensities.

† No association was found for ischemic lesions in the brainstem, large hemorrhages and sinus thrombosis.

‡ Pasquier scale ≥ 2 .

* Reference Fazekas score ≤ 1 .

a. $P < 0.05$; b. $P < 0.001$.

6

CHANGES IN WHITE MATTER MICROSTRUCTURE SUGGEST AN INFLAMMATORY ORIGIN OF NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

Objective: To assess white matter (WM) and gray matter (GM) magnetization transfer ratio histogram peak heights (MTR-HPHs) in different subsets of patients with neuropsychiatric systemic lupus erythematosus (NP-SLE) who have unremarkable findings on 3T magnetic resonance imaging of the brain and to evaluate whether these values could be used to highlight different clinically suspected underlying pathogenic processes or identify the clinical NP-SLE status or whether they could be associated with a specific NP-SLE syndrome.

Methods: Sixty-four SLE patients with neuropsychiatric symptoms were included. The initial NP-SLE diagnosis and suspected underlying pathogenic process were established by multidisciplinary evaluation. The final diagnosis was made after also considering the disease course 6–18 months later. Thirty-three patients with central nervous system (CNS) NP-SLE and 31 SLE patients with neuropsychiatric symptoms unrelated to SLE (non-SLE-related NP) were included. Twenty SLE patients without neuropsychiatric symptoms and 36 healthy control subjects were included for comparison. Differences in the WM and GM mean MTR-HPHs and between the different NP-SLE subgroups (CNS NP-SLE diagnosis, NP-SLE phenotype [inflammatory or ischemic], and clinical changes after treatment) and the relationship to NP-SLE syndromes were evaluated.

Results: Patients with inflammatory NP-SLE had significantly lower WM MTR-HPHs than did the healthy controls, the SLE patients, and the non-SLE-related NP patients. Cognitive disorder, mood disorder and psychosis were related to lower WM MTR-HPH values and cerebrovascular symptoms to higher values. Furthermore, the mean MTR-HPHs in the WM increased when the clinical status of the NP-SLE patients improved.

Conclusion: Measurement of MTR-HPH of the WM has the potential to identify inflammatory NP-SLE with CNS involvement. This finding underscores the usefulness of this technique for the detection of cerebral changes in NP-SLE patients and for the assessment of clinical changes after treatment.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by acute or chronic inflammation of multiple organs (1). Nervous system involvement in SLE, which is referred to as neuropsychiatric SLE (NP-SLE), leads to a broad, nonspecific, and heterogeneous group of NP manifestations (1,2). In 1999, the American College of Rheumatology (ACR) published a consensus document describing the diagnostic and exclusion criteria for 19 NP-SLE syndromes (3). Although widely used, its effectiveness is limited and NP-SLE remains a diagnosis per exclusion. Thus, in clinical practice, clinical suspicion of a certain pathogenic process underlying the clinical symptoms drives the therapeutic choice in these patients (4–6).

Two main underlying pathophysiologic processes have been described in NP-SLE, based on pathologic changes in humans and on findings in animal models. The inflammatory process (inflammatory NP-SLE) has been associated with dysfunction due to pathogenic antibodies and a disrupted blood–brain barrier, while the thrombotic process (ischemic NP-SLE) has been associated with focal neurologic deficits that can be attributed to interruption of blood flow in a specific brain region (5–7). Consistent with the suspected mechanism, therapy will be directed at the inflammation, with the use of immunosuppressive medications, or at the ischemia, with the use of antiaggregant and/or anticoagulant medications. These two phenotypes can also coexist.

So far, both the characterization of a certain NP-SLE phenotype and the correct attribution of NP events to SLE or to an alternative cause remain a challenge (8). None of the diagnostic tests currently used in clinical practice is specific for any NP-SLE manifestation or phenotype. Although magnetic resonance imaging (MRI) is the neuroimaging technique of choice in NP-SLE, this technique yields unremarkable findings in a significant proportion of patients, independently of the NP-SLE syndrome and its severity (8,9). There is thus an imperative need for radiologic techniques that help in the diagnostic process of NP-SLE and in the identification of NP-SLE phenotypes (2).

Magnetization transfer imaging (MTI) is a quantitative MRI technique known to be useful in the detection of cerebral abnormalities in brain tissue that looks normal on conventional MRI. This technique is based on the application of off-resonance radiofrequency pulses. Measurement of signal intensity with and without the application of these pulses allows the calculation of an index called the magnetization transfer ratio (MTR), which indirectly reflects the integrity of macromolecular structures (e.g., myelin) that exchange magnetization with the surrounding water (10,11). Among all of the MTI parameters, the histogram peak height (HPH), or the proportion of brain pixels at the most common MTR value, is the most informative parameter in NP-SLE without explanatory MRI findings. These values have been used as a quantitative estimate of tissue microstructural integrity in NP-SLE (12,13).

In preliminary investigations, Bosma and co-workers (14,15) observed a significantly lower whole-brain MTR-HPH in both active and past NP-SLE when compared with healthy controls. Those authors found an association between MTR-HPH and neurocognitive impairment and suggested that neuronal dysfunction may underlie central nervous system (CNS) involvement in NP-SLE (16). It has also been demonstrated that SLE patients with a history of NP had markedly lower gray matter (GM) MTR-HPHs than did healthy controls (17). Emmer and coworkers (18) showed how decreased whole-brain MTR-HPHs in patients with active NP-SLE increased when the clinical status improved, underscoring the possible partial reversibility of the previously observed abnormalities. Those authors also showed that in NP-SLE, there is a relationship between MTR-HPHs and neuronal impairment, as revealed by other quantitative neuroimaging techniques, such as diffusion-weighted imaging and proton magnetic resonance spectroscopy (13,19).

Despite these promising data, MTI has been applied only in a limited number of patients. The above-mentioned findings have never been reproduced in a NP-SLE cohort assessed through a multidisciplinary approach and followed prospectively. Prospective follow-up is essential for a diagnosis of NP-SLE. In the acute clinical setting, recognizing the cause of NP-SLE can be difficult, whereas at follow-up, the diagnosis can be assessed more reliably since the clinical course and response or failure to treatment provide diagnostic information.

The purposes of our study were to assess white matter (WM) and GM MTR-HPHs in a well-defined, prospectively followed cohort of SLE patients with NP symptoms that were either related or unrelated to SLE, to investigate whether these parameters may highlight different pathogenic NP-SLE processes (inflammatory or ischemic), and to reproduce previous findings published by our group in an evaluation of whether these parameters indicate the clinical NP-SLE status before and after treatment and whether they are related to different NP-SLE syndromes.

PATIENTS AND METHODS

Data source and population

All patients were admitted for a 1-day period to the Leiden University Medical Center. Our hospital serves as a national referral center for NP-SLE in the Netherlands. From September 1, 2007 through March 31, 2012, a total of 183 patients suspected of having NP involvement due to SLE were evaluated in the Leiden NP-SLE clinic. All patients underwent a standardized multidisciplinary medical examination, as well as extensive neuropsychological testing, serologic assessment, and brain MRI. Patients were classified according to the ACR 1982 revised criteria for SLE (20,21). SLE disease activity was determined with the use of the

Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (22). Irreversible damage due to SLE was assessed with the Systemic Lupus International Collaborating Clinics (SLICC)/ACR damage index (SDI) (23). The SLEDAI-2K and SDI values were calculated both with and without NP manifestations. Soon after evaluation, a consensus meeting took place. Further descriptions of the multidisciplinary evaluation and laboratory examination are available elsewhere (6,24). All patients were closely monitored by the referring physician and reevaluated by our group 6–18 months after the first visit. Twenty SLE patients without NP symptoms and 36 age-matched healthy control subjects were also included in this study. Patients over the age of 70 years were excluded. Written informed consent was obtained from all patients. The study was approved by the local medical ethics committee and was carried out in compliance with the Declaration of Helsinki.

NP-SLE subgroups

Diagnosis of NP-SLE was made by multidisciplinary consensus, and NP diagnoses were classified according to the ACR 1999 definitions of NP-SLE (3,20,21). More than 1 NP diagnosis per patient was possible. We included in the NP-SLE group only patients with at least 1 NP-SLE syndrome involving the CNS. For each NP-SLE patient, a suspected pathogenic mechanism was also assessed. We differentiated between inflammatory and ischemic NP-SLE, as discussed above. Both inflammatory and ischemic phenotypes could coexist in the same patient. Changes in the clinical NP status between the first and second visits were assessed 6–18 months later and were classified as worse, stable, or improved by multidisciplinary consensus (rheumatology [C-MC, TWH, and GMS-B], neurology [NDK], psychiatry [NJvdW], neuropsychology [HAM], and neuroimaging [BE and MAVB]). In an important subgroup of SLE patients, the NP symptoms were explained by another diagnosis. These SLE patients with NP symptoms unrelated to SLE (non-SLE-related NP) were considered a different subgroup. During follow-up, none of the patients in the 2 groups with NP symptoms ($n = 64$) developed new NP symptoms.

MRI protocol and scoring

All patients underwent brain MRI following the same protocol and using the same scanner on a regular course of maintenance. All scans were performed on a 3-Tesla MRI scanner (Achieva, Philips Healthcare, Best, The Netherlands). The protocol included high-resolution T_1 -weighted, T_2 -weighted and fluid-attenuated inversion recovery (FLAIR) sequences, followed by a T_1 -weighted sequence after intravenous administration of gadolinium contrast agent. An experienced radiologist (BE), who was blinded to the clinical status of the patients, examined visually all MRIs for the presence of abnormalities and for its suitability for MTI. To avoid the influence of ischemic areas due to thromboembolic processes on our results, we excluded patients with radiological evidence of other than incidental small ($> 5\text{mm}$)

infarctions and moderate atrophy measured by Pasquier scale (grade > 2; widened sulci, volume loss of the gyri). This scale, the most used visual rating scale (scores 0-3) for cortical atrophy, considers the volume of the gyri and width of the sulci (25). Subsequently, the differential diagnosis of ischemic NP-SLE without macroscopic MRI abnormalities included still cerebrovascular disease but also demyelinating syndromes and complex migraines.

MTI protocol

MTI-scans were performed using the same acquisition parameters for all NP-SLE, NP-non-SLE and SLE patients and HC. MTR data were obtained by using a 3-dimensional gradient echo sequence with an echo repetition/time of 100/11 msec and a low flip angle of 9°, to achieve minimal T_1 -weighting. Twenty slices of 7.2mm thickness were acquired in an axial orientation, with a field of view = $224 \times 180 \times 144 \text{ mm}^3$ and acquisition matrix = 224×210 (voxel size $0.875 \times 0.875 \text{ mm}^2$). To reduce acquisition time, segmented Echo-Planar Imaging (EPI) was applied, with 13 k-space profiles collected per excitation pulse (EPI factor 13). Two consecutive sets of axial images were acquired. The first set was performed in combination with a radiofrequency saturation pulse and the second without. Total scanning time was 1 minute and 8.3 seconds.

Image processing

For post-processing of magnetization transfer images, all images were transferred to an offline Linux workstation. All MTR processing steps were performed using software from the Oxford University Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) software library (FSL) (26). MTR was defined as flows:

$$\text{MTR} = ([M_0 - M_s]/M_0) \times 100$$

where M_s represents the signal intensity of voxels with saturation; and M_0 , the signal intensity of voxels without saturation. Skull stripping was performed using FSL BET (27). A detailed description of the segmentation process based on T_1 -weighted image and the way in which the resulting tissue masks were applied to the original MTR maps to calculate the tissue MTR maps (WM and GM) has been previously reported (13). To avoid the partial-volume effect of cerebrospinal fluid (CSF) at the tissue borders, the resulting maps were eroded in plane. From the remaining voxels, only those for which the probability of belonging to WM > 85% and GM > 80% were considered for the histogram analysis. All parenchyma segmentation was based on hard binary segmentations of GM and WM. All images were inspected visually to confirm adequate extraction of intracranial contents.

MTR histogram analysis

From the MTR maps, WM and GM MTR histograms were created with 100 bins and a bin size of 1. The first bin was excluded since it contains the voxels with an intensity of zero. The remaining 99 bins were taken into account for the subsequent calculations. MTR histograms were normalized for intracranial volume by dividing the number of voxels for each MTR value by the total number of CSF, WM and GM voxels. The corresponding peak height (PH) and peak location (PL) were calculated for WM and GM based on each normalized histogram using an in-house Matlab ® code. PL is an indicator of which MTR value is occurring more often. PH is a measure of the voxels fraction found to have the MTR value of the peak location. None of the WM or GM HPHs were used for clinical considerations.

Statistical analysis

The statistics included as primary dependent measures were the HPHs from the segmented WM and GM. Both were normally distributed. Equality of variances in WM and GM HPHs between NP-SLE, NP-non-SLE, SLE patients and controls was assessed using Levene's test. Between-group differences on WM and GM HPHs were evaluated using one-way-ANOVA's (pairwise comparisons). In the events of unequal variances, appropriate adjustments according to Tamhane's procedure in the pairwise comparisons of the means were performed. Analysis of covariance was performed to analyze the influence of disease duration, SLEDAI-2K, SDI, smoking status, hypertension and anticardiolipin antibodies (aCL) on the differences on mean PH values between groups. The association between NP-SLE syndromes and HPHs values was assessed by independent T-test analysis in every NP-SLE syndrome present in > 5 patients taking into account a possible inequality of variances. Paired-samples t-test was performed to test for significant mean HPHs differences before and after treatment of active NP-SLE patients. Statistical analysis was performed with SPSS version 20.0 for Windows (IBM SPSS statistics, Chicago, IL, USA).

RESULTS

Patient selection and characterization

From all evaluated patients, 135 (73.8%) fulfilled the revised ACR criteria for SLE. In 59 patients (43,7%) of these patients a diagnosis of CNS NP-SLE was established in the second visit, whereas in the remaining patients the NP complaints were not directly attributed to SLE. After MRI evaluation, a total of 33 patients with CNS NP-SLE and 31 NP-non-SLE patients were suitable for our MTR study. The rest of the patients were excluded due to the presence of abnormalities in the conventional MRI. **Table 1** shows clinical characteristics and autoantibody profiles of the study subjects at the time of the first MRI. SLEDAI-2K with and without NP symptoms and SDI with NP symptoms were significantly higher in the NP-

SLE group. No differences were found for SDI without NP symptoms. Among the patients diagnosed with CNS NP-SLE, 22 were diagnosed with inflammatory NP-SLE and 11 with ischemic NP-SLE. Fifty-four different ACR NP syndromes were established.

White and grey matter MTR peak heights and NP-SLE diagnosis

The mean and standard deviation (SD) of the WM and GM MTR-HPHs and the mean differences between the study groups are respectively summarized in **Table 3** and **Table 4**. NP-SLE patients with CNS involvement had significantly lower WM MTR-HPH than HC ($P < 0.001$) and SLE patients ($P = 0.001$). No differences were found between NP-SLE and NP-non-SLE ($P = 0.114$). NP-non-SLE had significantly lower WM MTR-HPH than HC ($P < 0.001$). After adjustment with Tamhane's procedure no statistically differences were found between NP-non-SLE and SLE patients ($P = 0.063$). Furthermore, no statistically significant differences were found for WM when SLE and HC were compared.

We did not find any mean GM MTR-HPH difference between the subgroups. Control for differences attributable to disease duration, SLEDAI-2K, SDI, smoking status, hypertension and aCL did not reveal any significant influence on previous calculations. **Figure 1** shows the mean WM MTR histograms after correction for intracranial volume for all the NP-SLE, NP-non-SLE, SLE patients and HC.

Table 1 Characteristics of the study subjects

	NPSLE (n = 33)	NP-non-SLE * (n = 31)	SLE ** (n = 20)	Healthy controls (n = 36)
Age, mean \pm SD years	37.2 \pm 13.3	39.4 \pm 14.9	41.1 \pm 11.1	40.1 \pm 11.8
Sex, no. female/male	29/4	28/3	18/2	32/4
SLE disease duration, mean \pm SD years	5.2 \pm 5.9	7.2 \pm 7.3	8.8 \pm 5.9	–
Neuropsychiatric symptoms duration, mean \pm SD years	1.2 \pm 2.7	2.7 \pm 3.3	–	–
SLEDAI-2K without NP	6.8 \pm 4.4	4.3 \pm 3.2 ^b	2.7 \pm 2.4 ^a	–
SLEDAI-2K with NP	13.6 \pm 5	4.3 \pm 3.2 ^a	2.7 \pm 2.4 ^a	–
SDI without NP	1.4 \pm 1.2	1 \pm 1.1	1.2 \pm 1.2	–
SDI with NP	2.2 \pm 1.4	1.2 \pm 1.1 ^a	1.2 \pm 1.2 ^b	–
ACR 1982 criteria for SLE †				
Malar Rash	16 (48.5)	14 (45.2)	11 (55)	–
Discoid rash	2 (6.1)	6 (19.4)	5 (25)	–
Photosensitivity	10 (30.3)	15 (48.4)	11 (55)	–
Mucosal ulcers	8 (24.2)	9 (29)	12 (60)	–
Arthritis	25 (75.7)	20 (64.5)	18 (90)	–
Serositis	9 (27.3)	10 (32.2)	3 (15)	–
Renal disorder	9 (27.3)	9 (29)	4 (20)	–
Neurological disorder	13 (39.4)	0 (0)	0 (0)	–
Hematologic disorder	17 (51.5)	14 (45.2)	15 (75)	–
Immunologic disorder	29 (87.9)	21 (67.7)	18 (90)	–
Positive ANA	31 (93.9)	30 (96.8)	20 (100)	–
Autoantibodies and complement †				
aCL IgG	8 (24.2)	5 (16.1)	2 (10)	–
aCL IgM	1 (3)	2 (6.5)	2 (10)	–
LAC	13 (39.4)	5 (16.1)	3 (15)	–
Antinuclear antibody	29 (87.9)	24 (77.4)	18 (90)	–
Anti-dsDNA	13 (39.4)	9 (29)	9 (45)	–
ENA	16 (48.5)	16 (51.6)	8 (40)	–
Anti-SSA	9 (27.3)	11 (35.5)	6 (30)	–
Anti-SSB	3 (9.1)	6 (19.4)	2 (10)	–
Anti-RNP	8 (24.2)	3 (9.7)	4 (20)	–
Anti-Sm	6 (18.2)	3 (9.7)	4 (20)	–
C1q low	3 (9.1)	1 (3.2)	1 (5)	–
C3 low	13 (39.4)	10 (32.3)	3 (15)	–
C4 low	12 (36.4)	6 (19.4)	5 (25)	–

aCL: anticardiolipin antibodies; ACR: American College of Rheumatology; ANA: antinuclear antibody; LAC: Lupus anticoagulant; NP: neuropsychiatric symptoms; NPSLE: neuropsychiatric systemic lupus erythematosus; SLE: systemic lupus erythematosus; SDI: systemic lupus international collaborating clinics (SLICC)/American College of Rheumatology damage index; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000.

* SLE patients with NP complaints non associated with CNS involvement due to SLE

** SLE patients without NP complaints

† Number and percentage per group

a. $P < 0.001$ when compared with NPSLE

b. $P < 0.05$ when compared with NPSLE

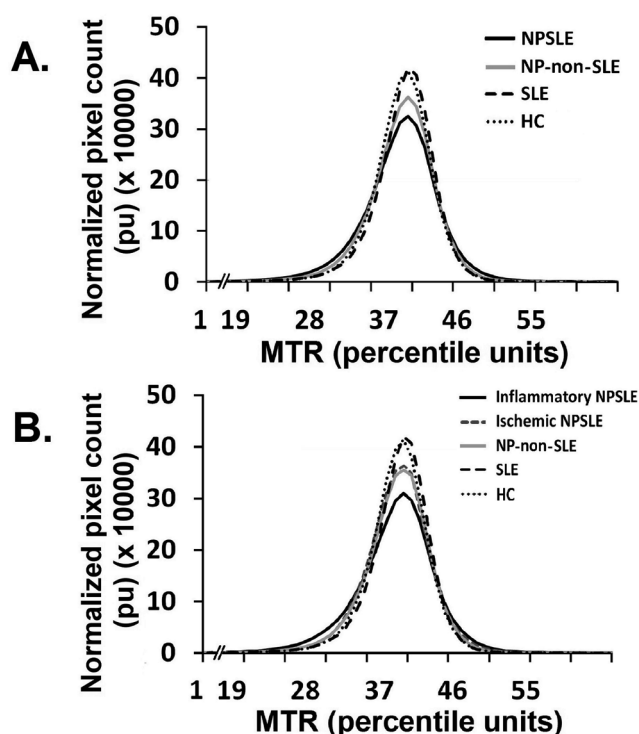


Figure 1. Average white matter magnetization transfer ratio (MTR) histograms. Mean MTR histograms after correction for intracranial volume are shown in A, patients with neuropsychiatric systemic lupus erythematosus (NPSLE), patients with NP symptoms unrelated to the underlying SLE (non-SLE-related NP), SLE patients without NP symptoms, and healthy control (HC) subjects, as well as in B, patients with inflammatory NPSLE, ischemic NPSLE, non-SLE-related NP, SLE patients without NP symptoms, and healthy control subjects. pu = percentage units.

White and grey matter MTR peak heights and NP-SLE phenotypes

The mean and SD of the WM and GM MTR-HPHs are presented in **Table 3**. The mean differences between the study groups are presented in **Table 4**. Patients with inflammatory NP-SLE had significantly lower WM MTR-HPH when compared with HC (WM $P < 0.001$), SLE (WM $P < 0.001$) and NP-non-SLE (WM $P = 0.023$). Moreover, inflammatory NP-SLE had a significantly lower WM MTR-HPH when compared with ischemic NP-SLE ($P = 0.001$). No statistically significant differences were found for WM when we compared ischemic NP-SLE with HC, NP-non-SLE or SLE. Inflammatory NP-SLE had also significantly lower GM MTR-HPH when compared with SLE ($P = 0.044$) but we did not find other differences when compared with other subgroups. We did not find any statistically significant difference for GM when ischemic NP-SLE patients were compared with HC, NP-non-SLE and SLE. Control for differences attributable to disease duration, SLEDAI-2K, SDI, smoking status, hypertension and aCL did not reveal any significant influence on previous calculations. WM MTR histograms in the 5 study groups are shown in **Figure 1**.

Table 2. Comparison of white matter and grey matter MTR-HPHs in the study groups *

		WM MTR-HPH §	GM MTR-HPH §
Healthy controls	36	43.37 ± 5.11	10.01 ± 2.51
SLE †	20	42.74 ± 6.22	10.02 ± 1.92
NP-non-SLE ‡	31	38.35 ± 4.64	9.81 ± 3.68
NPSLE	33	34.62 ± 7.55	8.56 ± 3.31
Phenotype			
Inflammatory NPSLE	22	32.22 ± 7.76	7.71 ± 3.25
Ischemic NPSLE	11	39.42 ± 4.21	10.25 ± 2.85

CNS: central nervous system; GM: grey matter; MTR-HPH: magnetization transfer ratio histogram peak height; NPSLE: neuropsychiatric SLE; SLE: systemic lupus erythematosus; WM: white matter.

* Values are the mean ± standard deviation.

† SLE patients without neuropsychiatric complaints

‡ SLE patients with neuropsychiatric complaints non associated with CNS involvement due to SLE

§ Peak height values were multiplied by 10,000 for readability

Table 3. Mean differences after Tamhane procedure of the WM and GM MTR-HPHs between the study groups *

	WM Peak height	GM Peak height
NPSLE diagnosis		
NPSLE – Healthy controls	–8.74 (0.000) † [–13.02 to –4.47]	–1.45 (0.247) [–3.39 to 0.48]
NPSLE – SLE	–8.12 (0.001) § [–13.38 to –2.85]	–1.64 (0.150) [–3.61 to 0.32]
NPSLE – NP-non-SLE	–3.73 (0.114) [–7.98 to 0.51]	–1.24 (0.654) [–3.63 to 1.14]
NP-non-SLE – Healthy controls	–5.01 (0.000) † [–8.24 to –1.77]	–0.21 (1.000) [–2.35 to 1.93]
NP-non-SLE – SLE	–4.39 (0.063) [–8.93 to 0.16]	–0.39 (0.997) [–2.56 to 1.76]
SLE – Healthy controls	–0.62 (0.999) [–5.19 to 3.94]	0.19 (1.000) [–1.45 to 1.84]
NPSLE phenotype		
NPSLE inflammatory – Healthy controls	–11.14 (0.000) † [–16.74 to –5.54]	–2.29 (0.073) [–4.71 to 0.12]
NPSLE inflammatory – SLE	–10.52 (0.000) † [–16.93 to –4.11]	–2.48 (0.044) § [–4.93 to –0.04]
NPSLE inflammatory – NP-non-SLE	–6.13 (0.023) § [–11.71 to –0.55]	–2.09 (0.296) [–4.91 to 0.72]
NPSLE inflammatory – NPSLE ischemic	–7.19 (0.001) § [–11.36 to –3.02]	–2.53 (0.276) [–5.96 to 0.89]
NPSLE ischemic – Healthy controls	–3.94 (0.165) [–8.75 to 0.86]	0.24 (1.000) [–2.89 to 3.37]
NPSLE ischemic – SLE	–3.32 (0.607) [–9.05 to 2.41]	0.47 (1.000) [–3.11 to 3.19]
NPSLE ischemic – NP-non-SLE	1.06 (0.999) [–3.73 to 5.86]	0.44 (1.000) [–2.92 to 3.81]

GM: grey matter; HC: healthy controls; MTR-HPH: magnetization transfer ratio histogram peak height; NPSLE: neuropsychiatric SLE; SLE: systemic lupus erythematosus; NP-non-SLE: systemic lupus erythematosus with neuropsychiatric complaints non-SLE related; WM: white matter.

* Mean difference (P-value) [95% CI]

† Indicates significance level at $P < 0.001$

§ Indicates significance level at $P < 0.05$

White and grey matter MTR peak heights and NP-SLE syndromes

Independent T-test analysis was performed for every NP-SLE syndrome present in > 5 patients. Patients with cerebrovascular disease (n = 11), psychosis (n = 8), headache (n = 8), seizure (n = 5), cognitive disorder (n = 9) and mood disorder (n = 10) were analyzed individually. Psychosis was associated with a lower WM MTR-HPH ($P = 0.033$) and GM MTR-HPH ($P = 0.029$). We also found an association between a lower WM MTR-HPH and cognitive disorder ($P = 0.047$) and mood disorder ($P = 0.025$). We did not find any association between GM MTR-HPHs and cognitive disorder or mood disorder. Furthermore, cerebrovascular disease was associated with higher WM MTR-HPH ($P = 0.006$). No associations were found between MTR-HPHs and patients with headache or seizure.

White matter MTR peak heights and clinical changes

From all the twenty NP-SLE patients considered to have active CNS disease during the first visit, eleven patients improved after treatment, seven were classified as stable and 2 patients deteriorated. Mean histogram and standard deviation WM MTR-HPH of all patients at first visit was 31.51 ± 7.83 . On the follow-up visit these values increased and mean MTR-HPH was 39.07 ± 6.56 . WM MTR histograms after correction for intracranial volume before and after treatment are shown in **Figure 2**. In all NP-SLE patients that clinically improved, the mean WM MTR-HPH increased in 9.81 ± 5.94 [range 5.81 to 13.81] ($P < 0.000$). WM MTR-HPH mean difference of patients classified as stable on the second visit were 2.48 ± 4.65 [range 1.81 to 6.79] ($P = 0.207$). In the two patients who deteriorated, a decrease on the WM MTR-HPH between the first and second MRI was observed -10.32 ± 0.41 [range -14.01 to -6.63] ($P = 0.018$).

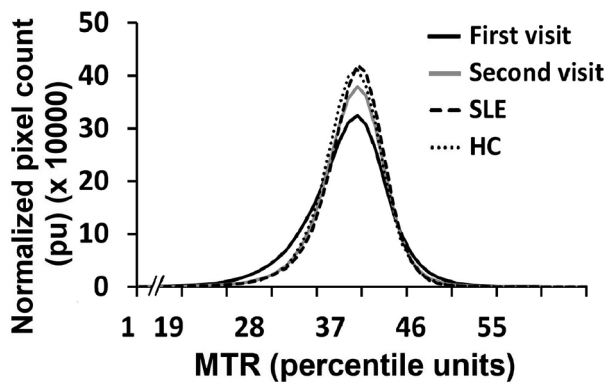


Figure 2. Average magnetization transfer ratio histograms of white matter from active NPSLE patients on the first visit and after treatment on the second visit. SLE patients without neuropsychiatric complaints and healthy controls (HC) on the basal visit are also included for comparison.

DISCUSSION

This study is the first to show that NP-SLE patients with an inflammatory phenotype have significantly lower WM MTR-HPHs than do ischemic NP-SLE, non-SLE-related NP, or SLE patients or healthy controls. We also found that WM MTR-HPH is sensitive to clinical changes. Based on these findings, we propose that the WM MTR-HPH is a potentially valuable tool for use in the diagnosis and follow-up of inflammatory NP-SLE.

Inflammatory NP-SLE is thought to reflect neuronal dysfunction mediated by inflammatory factors, autoantibodies, and increased SLE disease activity. Apart from global and localized ischemic changes, histopathologic data in NP-SLE show parenchymal edema, glial hyperplasia, and diffuse neuronal/axonal loss (7). It has been hypothesized that MTR changes are associated with all of these findings and may thus also explain our results (13,18). In multiple sclerosis, MTR abnormalities have been described as a useful tool for assessing disease burden and evaluating disease progression (28). However, demyelination is not a primary phenomenon in NP-SLE, and other mechanisms may play a more important role in these MTR changes (8). The fact that the WM MTR-HPHs in patients with ischemic NP-SLE, mainly seen in those with cerebrovascular symptoms, were lower than those in the healthy controls and significantly higher than those in patients with inflammatory NP-SLE may suggest cumulative chronic damage of the brain, as reported previously (13,16). Furthermore, mean MTR-HPHs at the second visit were, on average, closer to those in ischemic NP-SLE patients, probably reflecting residual effects or WM-specific and irreversible changes in patients with past inflammatory NP-SLE.

To our knowledge, this is the first study in which prospective follow-up was performed in order to avoid misclassification of the putative cause of NP symptoms in SLE. This standardized assessment is the most appropriate reference standard for diagnosis so far (29). In addition, we were able to include patients with CNS involvement without remarkable abnormalities on MRI. These well-defined data are an additional benefit of our study. This study also reproduced some data previously published by our group.

We found that NP-SLE patients and non-SLE-related NP patients have, on average, significantly lower WM MTR-HPHs than do healthy controls. Furthermore, the WM MTR-HPHs in NP-SLE patients were significantly lower on average than those in SLE patients, but no differences were found between SLE patients and non-SLE-related NP patients. The usefulness of whole-brain parenchyma or segmented tissue MTR-HPHs for the differentiation of SLE patients with NP symptoms has previously been reported (13–15,17,30,31). Studies based on other quantitative radiologic techniques, such as proton magnetic resonance spectroscopy and diffusion tensor imaging, have demonstrated a loss of WM integrity in SLE

patients and non-SLE-related NP patients as compared with healthy controls (13,32–34). Using MTI, we found no differences between SLE patients and healthy controls, which may suggest that each technique identifies different aspects of the microstructural changes in the brains of SLE and NP-SLE patients. As previously reported, no differences between NP-SLE patients and non-SLE-related NP patients were found, probably because the NP-SLE group included both ischemic and inflammatory NP-SLE subgroups (13).

There may be 2 possible explanations for the lower WM MTR-HPH values in the non-SLE-related NP patients. Despite multidisciplinary assessment, we still might have misclassified some NP-SLE patients as having non-SLE-related NP. Additionally, the non-SLE-related NP group included a broad spectrum of active neurologic and psychiatric disorders, which may have influenced the MTR results, as lower MTR values have been previously reported in patients with behavioral, psychotic, and neurodegenerative disorders (35–37).

Cognitive dysfunction was associated with lower WM MTR-HPHs, as previously observed in other studies (13,16). We also found an association between psychosis and lower WM and GM MTR-HPHs, as well as between mood disorder and WM MTR-HPHs. In contrast, cerebrovascular disease was related to higher WM MTR-HPHs, and no associations for headache or seizure were noted. Cognitive dysfunction, psychosis, and mood disorder may share a similar pathogenic pathway as compared with other syndromes. However, these results may be related to the prevalence of certain syndromes and their activity at the time of MRI as well as to the heterogeneity of NP-SLE. As mentioned above, nonspecific microstructural changes of the brain tissue as measured by MTR have been found in several brain regions in patients with cognitive impairment, psychosis, and mood disorder (35–37).

As demonstrated previously (18), we have seen how brain involvement in patients with active NP-SLE with unremarkable findings on MRI is partially reversible when measuring WM MTR-HPHs. These values decreased or increased in parallel with the clinical status of the patients, as assessed by our multidisciplinary group. It has been suggested that these changes may be linked to the resolution or exacerbation of general inflammatory changes of the brain (7,18). It is unclear whether these MTR changes after treatment are associated with remyelination, as has been demonstrated in multiple sclerosis (18,38). Our data reinforce the idea that MTI, especially the MTR histogram analysis, may be a useful tool for evaluating disease progression and response to therapy.

Our results also show a lower GM MTR-HPH in patients with inflammatory NP-SLE as compared with those with SLE and a trend as compared with healthy controls. The difference between NP-SLE patients and healthy controls was previously reported by Steens and coworkers (17). The selective lowering of the GM MTR-HPH in patients with inflammatory

CNS NP-SLE without remarkable abnormalities on MRI may reveal GM-specific changes. However, these data should be viewed with caution, since several factors could affect these results. The presence of cortical atrophy, especially focal, has been observed in NP-SLE (8,9). Due to partial volume effects, the voxels analyzed in the parenchyma I cortex contain a mixture of GM, WM, and CSF. This may lead to a misclassification of those voxels as GM and, subsequently, to decreased GM MTR-HPHs. To avoid the effect of atrophy, we used the Pasquier scale for patient selection, as well as stringent thresholds for GM parenchyma analysis to reduce partial volume effects as much as possible without losing the representation of the segmented tissue type.

We were not able to reproduce other data previously published by our group in studies of a smaller number of patients. Steens and coworkers found an association between certain MTR values (WM and GM mean MTR and peak location) and positivity for IgM aCL, suggesting that these antibodies may be associated with diffuse brain involvement (17). This association between MTR values and aCL status was not further confirmed (13). We found no association between aCL and HPHs. Previously, an association between certain SLE criteria, such as arthritis and renal involvement, and MTR-HPHs was observed (13). In the present study, associations between HPHs and disease activity (SLEDAI-2K) were not found. We believe that our previous data may show false-positive associations based on the small sample size.

The main limitation of our study is the small number of patients per group and per syndrome. This is a generally recognized problem related to the low prevalence and the high heterogeneity of NP-SLE. We therefore cannot draw definite conclusions concerning the relationship between the MTR-HPH findings and NPS LE syndromes. Furthermore, due to matters of referral, some of the patients with inflammatory NP-SLE were evaluated in the NP-SLE clinic once they had started the immunosuppressive therapy. This may explain the higher variance in the NP-SLE group, and we believe that inflammatory NP-SLE would probably have shown lower values in comparison with other groups if none of these patients had received prior therapy.

A second limitation is that for research purposes, we selected patients with unremarkable findings on MRI, excluding a high proportion of patients to avoid the influence of thromboembolic processes. Our data can thus be extrapolated only to NP-SLE patients with unremarkable MRI findings, since the effect of the presence of infarcts and WM lesions on the MTR-HPHs values remains unknown. Another limitation of our study is the possible misclassification of inflammatory NP-SLE based on a good response to therapy, whereas the clinical response could have been the normal waxing and waning of the disease course or due to their inclusion in this group of nonspecific NP-SLE syndromes (headache, mood disorder, anxiety, and mild cognitive dysfunction). However, such misclassification would

lead to smaller differences between groups, and the real differences may therefore be even larger than we report here. A final limitation is that due to the impaired clinical status of some patients, we had to decrease the scanning time, which subsequently affected the resolution, resulting in partial volume effects, which may cause misclassification of GM and WM voxels.

In conclusion, this is the first study to demonstrate that WM MTR-HPHs might provide evidence of the presence of inflammatory NP-SLE. This study also confirmed the usefulness of this technique in the detection of cerebral changes in NP-SLE and in the assessment of clinical changes after treatment of patients with active disease. Moreover, a lower WM MTR-HPH was associated with cognitive dysfunction, mood disorder, and psychosis. Further studies are required to fully determine whether these data reflect the burden of SLE on the brain or whether they represent the severity of NP symptoms apart from the SLE. Our results are consistent with previous data reported by our group, thus broadening their significance. The findings of our study illustrate the value of MTR-HPH analysis as a potential radiologic biomarker that may help in the diagnostic process and follow-up of patients with NP-SLE and with the monitoring of future treatment trials.

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7

GLIAL AND AXONAL CHANGES IN SYSTEMIC LUPUS ERYTHEMATOSUS MEASURED WITH DIFFUSION OF INTRACELLULAR METABOLITES

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ABSTRACT

Systemic lupus erythematosus is an inflammatory autoimmune disease with multi-organ involvement. Central nervous system involvement in systemic lupus erythematosus is common and results in several neurological and psychiatric symptoms that are poorly linked to standard magnetic resonance imaging outcome. Magnetic resonance imaging methods sensitive to tissue microstructural changes, such as diffusion tensor imaging and magnetization transfer imaging, show some correlation with neuropsychiatric systemic lupus erythematosus (NP-SLE) symptoms. Histological examination of NP-SLE brains reveals presence of cerebral oedema, loss of neurons and myelinated axons, microglial proliferation and reactive astrogliosis, microinfarcts and diffuse ischaemic changes, all of which can affect both diffusion tensor imaging and magnetization transfer imaging in a non-specific manner. Here we investigated the underlying cell-type specific microstructural alterations in the brain of patients with systemic lupus erythematosus with and without a history of central nervous system involvement. We did so combining diffusion tensor imaging with diffusion-weighted magnetic resonance spectroscopy, a powerful tool capable of characterizing cell-specific cytomorphological changes based on diffusion of intracellular metabolites. We used a 7 T magnetic resonance imaging scanner to acquire T1-weighted images, diffusion tensor imaging datasets, and single volume diffusion-weighted magnetic resonance spectroscopy data from the anterior body of the corpus callosum of 13 patients with systemic lupus erythematosus with past NP-SLE, 16 patients with systemic lupus erythematosus without past NP-SLE, and 19 healthy control subjects. Group comparisons were made between patients with systemic lupus erythematosus with/without past NP-SLE and healthy controls on diffusion tensor imaging metrics and on diffusion coefficients of three brain metabolites: the exclusively neuronal/axonal N-acetylaspartate, and the predominantly glial creatine + phosphocreatine and choline compounds. In patients with systemic lupus erythematosus with past NP-SLE, significantly higher diffusion tensor imaging mean and radial diffusivities were accompanied by a significantly higher intracellular diffusion of total creatine ($0.202 \pm 0.032 \mu\text{m}^2/\text{ms}$, $P=0.018$) and total choline ($0.142 \pm 0.031 \mu\text{m}^2/\text{ms}$, $P=0.044$) compared to healthy controls ($0.171 \pm 0.024 \mu\text{m}^2/\text{ms}$, $0.124 \pm 0.018 \mu\text{m}^2/\text{ms}$, respectively). Total N-acetylaspartate, total creatine and total choline diffusion values from all patients with systemic lupus erythematosus correlated positively with systemic lupus erythematosus disease activity index score ($P=0.033$, $P=0.040$, $P=0.008$, respectively). Our results indicate that intracellular alterations, and in particular changes in glia, as evidenced by increase in the average diffusivities of total choline and total creatine, correlate with systemic lupus erythematosus activity. The higher diffusivity of total creatine and total choline in patients with NP-SLE, as well as the positive correlation of these diffusivities with the systemic lupus erythematosus disease activity index are in line with cytomorphological changes in reactive glia, suggesting that the diffusivities of choline compounds and of total creatine are potentially unique markers for glial reactivity in response to inflammation.

Systemic lupus erythematosus (SLE) is a female predominant autoimmune disease that affects multiple organs.(1) Central nervous system (CNS) involvement in SLE is common and results in several neurological and psychiatric symptoms. These symptoms are poorly characterized by standard magnetic resonance imaging (MRI), which appears normal in about 50% of patients with neuropsychiatric systemic lupus erythematosus (NP-SLE). Focal lesions and vascular infarcts, visible on MRI of patients with NP-SLE, are non-specific and often do not correlate with clinical outcome and with symptom severity.(2)

MRI methods sensitive to tissue microstructural changes, such as diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI), show diffuse white matter changes that correlate with the clinical status of patients with NP-SLE.(3-7) Histological examination of NP-SLE brains has revealed the presence of cerebral oedema, loss of neurons and myelinated axons, microglial proliferation and reactive astrogliosis, microinfarcts and diffuse ischemic changes, all of which can affect the image contrast in DTI and MTI.(8) Therefore, although clinically informative, due to the lack of specificity, these imaging modalities provide limited insight into the microstructural deficit in NP-SLE.

Magnetic resonance spectroscopy (MRS) reports on concentrations of cell-specific metabolites, and MRS studies have shown differences in the concentrations (relative to total creatine) of several brain metabolites, including significantly lower N-Acetylaspartate (NAA) and significantly higher choline and myo-inositol levels in patients with SLE and NP-SLE compared to healthy controls.(9,10) In addition, one study reported significantly lower NAA in SLE patients with high disease activity compared to those with low disease activity.(11) Although MRS provides cell-type specific information, it does not provide any structural information.

Diffusion-weighted magnetic resonance spectroscopy (DW-MRS) combines the cell-type specificity of MRS with the microstructural sensitivity of diffusion-weighted imaging (DWI), and allows studying cell- and compartment-specific properties of tissue microstructure by probing the diffusion of intracellular brain metabolites.(12,13) Of these metabolites, N-acetylaspartate typically co-measured with N-acetylaspartylglutamate (NAAG) ($\text{NAA} + \text{NAAG} = \text{tNAA}$) resides almost exclusively in neurons/axons; creatine and phosphocreatine ($\text{Cr} + \text{PCr} = \text{tCr}$), pivotal in aerobic cell energetics, are found in all neural cells, but their astrocytic concentration is twice their neuronal one, and soluble choline-containing compounds (tCho) are predominantly glial, with a glial/neuronal concentration ratio of 3:1.(14,15) The diffusion properties of these metabolites are strongly dictated by the structural and physiological features of their respective intracellular space, and thus provide a unique in-vivo probe for pathology affecting intracellular structures, such as ischemia, tumors, and axonopathy in multiple sclerosis (MS), as well as making accurate vivo cell-specific

characterization of tissue microstructure possible.(16-21)

In this study we utilize for the first time the sensitivity of DW-MRS to selectively report on axonal and glial microstructure (a) to investigate the underlying microstructural alterations in a normal appearing portion of the corpus callosum in the brain of SLE patients with and without history of NP-SLE and (b) to assess the relationship between DW-MRS indices and SLE activity in the patient population in this study. These studies were performed at ultrahigh field (7 Tesla) in order to obtain the sensitivity required for robust DW-MRS measurements.

MATERIALS AND METHODS

Human Subjects

Twenty-nine SLE patients (one male, 28 females, age: 43 ± 10 years) and 19 age- and sex-matched healthy volunteers (one male, 18 females, age: 41 ± 11 years) were included in the study. The study adhered to the Helsinki Declaration and was approved by the institutional review board of our institution. Written informed consent was obtained from all subjects prior to the study. Of 29 SLE patients, 13 had a history of NP-SLE and sixteen had no history. For convenience, patients with NP-SLE in the past are referred to as “patients with NP-SLE” throughout the text. All patients with SLE were diagnosed according to the 1982 revised American College of Rheumatology criteria.(22,23) All NP-SLE patients were diagnosed at the Leiden NP-SLE-clinic after a standardized multidisciplinary medical examination.(24) NP diagnoses were classified according to the 1999 American College of Rheumatology case definitions for NP-SLE syndromes.(25) The NP syndromes in our NP-SLE cohort included cerebrovascular disease (5 patients), seizures (3 patients), cognitive disorder (3 patients), movement disorder (1 patient), headache (2 patients), acute confusional state (3 patients), psychosis (1 patient), transverse myelitis (1 patient), polyneuropathy (1 patient), anxiety (1 patient), and radiculopathy (1 patient). In order to categorize the patients according to SLE disease activity, we calculated the systemic lupus erythematosus disease activity index 2000 (SLEDAI-2K) for each patient.(26) Permanent and irreversible damage due to SLE was assessed with the systemic lupus international collaborating clinics (SLICC)/American College of Rheumatology damage index (SDI).(27,28) SLE patients with a SLEDAI-2K ≥ 8 were considered to have high SLE activity and were categorized as SLE-active, while the remaining SLE patients were categorized as SLE-inactive.(11) The demographics of the study and the clinical characteristics of the SLE patients are shown in **(Table 1)**.

Table 1: Patient characteristics

	NPSLE patients (n=13)	SLE patients (n=16)	P
Age (years)	43 ± 8	42 ± 11	0,721
SLE disease duration (years)	12 ± 9	8 ± 5	0,178
SLEDAI-2K	7 ± 6	3 ± 2	0,006
SDI	2 ± 2	1 ± 1	0,241
Patient characteristics			
Antiphospholipid syndrome	3/13 (23%)	1/16 (6%)	0,223
Presence of auto-antibodies			
Antinuclear antibody	12/13 (92%)	14/16 (88%)	0,580
Anti-ENA	9/13 (70%)	7/16 (44%)	0,160
Anti-DNA	1/13 (8%)	6/16 (38%)	0,074
Anti-RNP	3/13 (23%)	3/16 (19%)	0,565
Anti-SSA	4/13 (31%)	7/16 (44%)	0,372
Anti-SSB	1/13 (8%)	2/16 (13%)	0,580
Anti-Smith	2/13 (15%)	2/16 (13%)	0,617
Anticardiolipine Autoantibodies	3/13 (23%)	1/16 (6%)	0,223
Lupus Anticoagulant	6/13 (46%)	2/16 (13%)	0,055
Anti-B2 Glycoproteine IgG	2/13 (15%)	0/16 (0%)	0,192
Presence of ACR criteria ever in disease course			
Malar rash	5/13 (38%)	8/16 (50%)	0,404
Discoid lupus	2/13 (15%)	0/16 (0%)	0,192
Photosensitivity	5/13 (38%)	7/16 (44%)	0,537
Ulcers	7/13 (54%)	7/16 (44%)	0,434
Arthritis	12/13 (92%)	9/16 (56%)	0,038
Serositis	4/13 (31%)	6/16 (38%)	0,507
Lupus nephritis	4/13 (31%)	5/16 (31%)	0,647
Neurological disorder	4/13 (31%)	0/16 (0%)	0,448
Hematologic disorder	6/13 (46%)	8/16 (50%)	0,566
Immunologic disorder	9/13 (70%)	13/16 (81%)	0,374
Antinuclear antibodies	13/13 (100%)	16/16 (100%)	0,374
Current medication			
Prednisone	9/13 (70%)	9/16 (56%)	0,372
Azathioprine	3/13 (23%)	6/16 (38%)	0,336
Methotrexate	1/13 (8%)	1/16 (6%)	0,704
Hydroxychloroquine	11/13 (85%)	12/16 (75%)	0,435
Mycophenolate mofetil	3/13 (23%)	2/16 (13%)	0,396

ACR = American College of Rheumatology; IgG = immunoglobulin G; SDI = systemic lupus international collaborating clinics (SLICC)/American College of Rheumatology damage index.

*P<0.05; ** P<0.01.

Data Acquisition

All subjects were scanned on a 7 tesla Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands) equipped with a 32-channel receive head coil (Nova Medical Inc., Wilmington, MA, USA). The scan protocol consisted of a short survey scan and a sensitivity encoding reference scan followed by (a) sagittal 3D T₁-weighted images (Field of view (FOV): 246×246×174 mm³, resolution: 0.85×0.85×1 mm³, repetition time/echo time (TR/TE): 4.00 /1.84 ms, total scan time: 1.59 min); (b) axial multislice diffusion tensor images (FOV: 224×224×150 mm³, resolution: 1.75×1.75×2.20 mm³, TR/TE: 10000/65 ms, 15 diffusion weighting directions with b = 1000 s/mm², total scan time: 3 min) and (c) single-volume diffusion-weighted spectroscopy scans (detailed protocol below).

DW-MRS Protocol

The DW-MRS sequence was based on the PRESS (Point Resolved Spectroscopy) sequence with bipolar diffusion-weighting gradients added on both sides of the 180° pulses. A 3cm³ volume of interest (VOI) (25 (AP) × 15 (RL) × 8 (FH) mm³) was positioned on the anterior body of the corpus callosum as shown in **Figure 1**. The diffusion-weighting gradients were applied in two directions: a right-left direction in the VOI frame, mostly parallel to the direction of the callosal fibers (direction [1,0,0]), and a direction mostly perpendicular to the callosal fibers (direction [0,-1,1]), as shown in **Figure 1A and B**. The center frequency was set to the tNAA singlet peak at 2.0 ppm. Water suppression was performed using two frequency-selective excitation pulses, each followed by a dephasing gradient before metabolite excitation. Pencil beam second-order shimming was performed, resulting in a typical tNAA line width of 10 Hz. A peripheral pulse unit was used in order to gate data acquisition to the cardiac cycle, thereby minimizing signal fluctuations due to cardiac pulsation. The parameters for DW-MRS acquisitions were: TE = 121 ms, TR = 3 cardiac cycles (about 3000 ms), cardiac trigger delay = 300 ms, number of time-domain points = 1024, spectral width = 3000 Hz, gradient duration (δ) = 37 ms, bipolar gap = 16 ms, diffusion time (Δ) = 60.5 ms with 5 different gradient amplitudes resulting in b-values of 212, 651, 1335, 2262, and 3462 s/mm² in the [1,0,0] direction and 440,1336, 2718, 4586, and 6945 s/mm² in the [0,-1,1] direction. The total number of spectra per diffusion condition was 32, resulting in a total scan time of 10~15 minutes. Following this scan, a shorter scan (fewer signal averages) with identical VOI position and diffusion conditions was performed without water suppression and with the center frequency set at the water resonance frequency. These spectra were used for eddy-current correction in the post-processing stage.

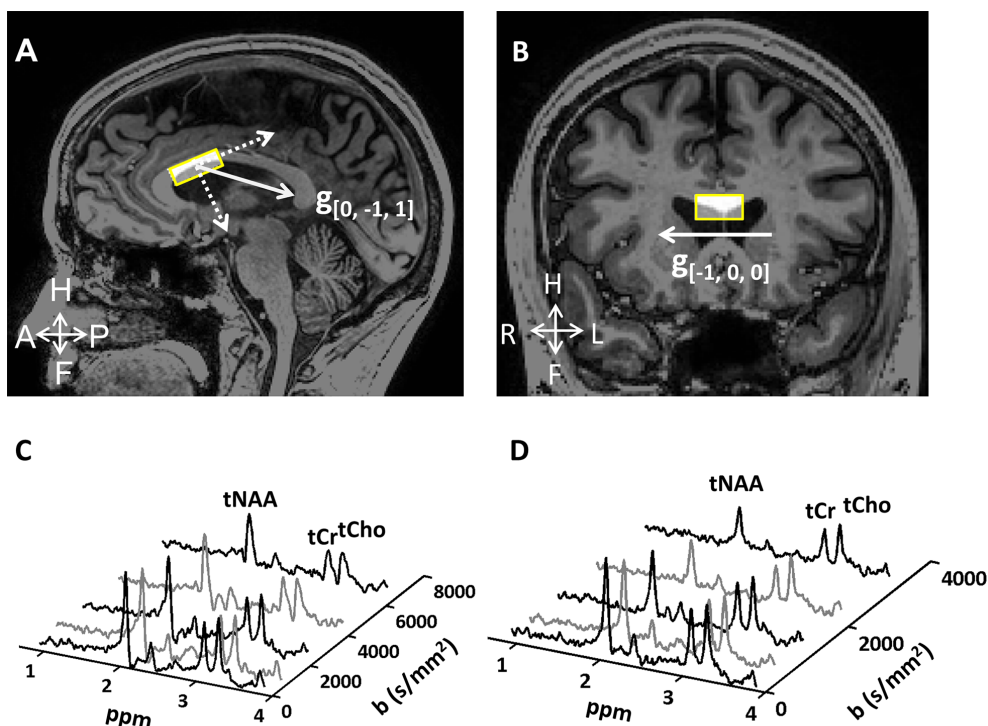


Figure 1. The position of the volume of interest in sagittal (A) and coronal (B) views. Gradients applied in directions approximately perpendicular (A) and parallel (B) to the callosal fibers are shown in solid lines. Typical spectra acquired with diffusion weighting in the $[0, -1, 1]$ and the $[1, 0, 0]$ directions are shown as a function of b-value in panels (C) and (D), respectively. Line broadening of 5 Hz was applied for display purposes.

DW-MRS Processing

All spectral pre-processing was performed with custom codes in MATLAB® release R2014b (Mathworks, Natick, MA, USA). Spectral preprocessing consisted of correcting DW-MRS data for eddy currents, zero-order phasing, correction of frequency drift for individual acquisitions, and removal of the residual water peak: averaged spectra were generated for each condition.(29) **Figure 1C and D** show typical sets of diffusion-weighted spectra obtained with diffusion-weighting in the $(1, 0, 0)$ direction (**Figure 1C**) and the $(0, -1, 1)$ direction (**Figure 1D**), respectively and from a healthy control subject. The resulting spectra were quantified with LCModel.(30) Cramér–Rao lower bound (CRLB) values were used to evaluate the quality of the spectra for each diffusion condition, and the acceptance threshold for DW-MRS data inclusion was set at $CRLB < 20\%$. Based on this, data sets from one NP-SLE subject and one SLE subject were excluded from the tNAA analysis.

The LCModel spectral estimates were used to calculate the diffusivity (D_{par}) along the [1,0,0] direction (roughly parallel to the callosal fibers) and diffusivity (D_{perp}) along the [0,-1,1] direction (roughly perpendicular to the callosal fibers) for tNAA, tCr and tCho. These were calculated by performing a linear fit of the natural logarithm of the DW-MRS signal amplitudes as a function of the diffusion weighting value b , assuming a monoexponential decay of the signal as a function of b in each direction:

$$\ln\left(\frac{S_{b,i}}{S_{b_0}}\right) = -b_i \cdot D_i$$

where $S_{b,i}$ is the measured signal in direction i , S_{b_0} is the signal without diffusion weighting, b_i is the value of b in the direction i , and D_i is the calculated diffusion coefficient for direction i . Even though it is possible that the metabolite diffusion-weighted signal decay displays non-monoexponential behavior at very high values of b , our previous work has shown that in the range of b values used in this study the assumption of monoexponentiality is valid and diffusivity values are reproducible.(13,29) An average of D_{par} and D_{perp} was calculated to assess the average diffusivity (D_{avg}) for tNAA, tCr and tCho. The quality of the linear fittings was evaluated via calculation of the coefficient of determination and an acceptance threshold was set at 75%, leading to exclusion of D_{avg} (tCr) values obtained from two patients with NP-SLE and one patient with SLE.

Image Processing

DTI volumes were motion-corrected with ExploreDTI and further processed with the DTI toolbox (31) of the FMRIB Software Library (FSL release 5.0, <http://www.fmrib.ox.ac.uk/fsl/>) to obtain the following DTI measures for each subject: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). These DTI metrics were further analyzed with tract based spatial statistics (TBSS).(32) Statistical differences between NP-SLE patients, SLE patients and HC were assessed in FA-MNI152 standard space using 5000 permutations and were corrected for multiple comparison based on threshold-free cluster enhancement(Winkler *et al.* 2014). One SLE patient data set was excluded due to poor registration to FA-MNI152.

T_1 -weighted images were used for tissue segmentation within the VOI.(29) Fractional anisotropy maps were registered to the T_1 -weighted image of the same subject first by affine transformation using FSL FLIRT and subsequently by non-rigid transformation using FNIRT. (33-35) The inverse transformation matrices generated were used to register the DW-MRS VOI to the DTI space. Subsequently, the registration procedure in TBSS was applied to transform each VOI to MNI152 space.

T_1 -weighted volumes were further processed in FreeSurfer (<http://surfer.nmr.mgh.harvard.edu/>) and the intracranial volume, total brain volume, center corpus callosum volume and mid-anterior corpus callosum volume were calculated for each subject. To evaluate whole brain and callosal atrophy due to SLE and NP-SLE, total brain volume, center corpus callosum volume and mid-anterior corpus callosum volume were normalized according to the intracranial volume of the same subject.

Statistical Analyses

Patients with NP-SLE and those with SLE were compared with respect to demographic characteristics, presence of autoantibodies and ACR criteria and current medication using chi-square tests and ANOVA or Mann-Whitney tests when appropriate. Primary dependent measures included in the statistics were $D_{avg}(tNAA)$, $D_{avg}(tCr)$, $D_{avg}(tCho)$. Shapiro-Wilk test was used to examine the distribution of variables, and resulted in all being normally distributed. Equality of variances of the different groups was assessed using Levene's test. Between-group differences on all D_{avg} values were evaluated using one-way ANOVAs (pairwise comparisons). $P < 0.05$ was considered to represent statistically significant differences. Bonferroni's correction was used to correct for multiple comparisons. Analysis of covariance was performed to analyse the influence of age and SLEDAI-2K on the differences of mean D_{avg} values between groups. As SLEDAI-2K and SDI scores were not normally distributed, correlations between the metabolite D_{avg} measurements and SLEDAI-2K score and SDI score were evaluated with Spearman's rank correlation. Correlations between SLE duration and D_{avg} values were assessed with Pearson's correlation test. All statistical analyses were performed with SPSS version 20.0 for Windows (IBM SPSS statistics, Chicago, IL, USA). Scattered plots were generated using GraphPad Prism 5 for windows, version 5.01, GraphPad Software, USA.

RESULTS

DW-MRS results

Average metabolite D_{avg} values for the NP-SLE, SLE and healthy control groups are shown in **Table 2** and group D_{avg} data for the three population groups are displayed in **Figure 2**. When all three groups were compared with ANOVA with age included as a covariate, significant differences were found in $D_{avg}(tCho)$ ($P = 0.006$) and $D_{avg}(tCr)$ ($P = 0.030$). No significant differences were found in $D_{avg}(tNAA)$ among the groups.

Table 2: Metabolite D_{avg} values for NPSLE patients, SLE patients and healthy controls.

	NPSLE patients (n = 13)	SLE patients (n = 16)	Healthy controls (n=19)	% increase in NPSLE vs HC
$D_{avg}(tNAA) \mu m^2/s$	0.24 ± 0.02	0.23 ± 0.02	0.23 ± 0.02	7%
$D_{avg}(tCr) \mu m^2/s$	$0.20 \pm 0.03^{**}$	0.18 ± 0.03	0.17 ± 0.02	18%
$D_{avg}(tCho) \mu m^2/s$	$0.14 \pm 0.03^*$	0.13 ± 0.02	0.12 ± 0.02	14%

*p-value<0.05 versus healthy controls **p-value<0.01 versus healthy controls

Pairwise comparisons showed that in patients with NP-SLE, $D_{avg}(tCr)$ and $D_{avg}(tCho)$ were significantly higher than in healthy controls after correction for age (mean $D_{avg}(tCr)$ in NP-SLE = $0.202 \pm 0.032 \mu m^2/ms$, mean $D_{avg}(tCr)$ in healthy controls = $0.171 \pm 0.024 \mu m^2/ms$, $P = 0.018$ and mean $D_{avg}(tCho)$ in NP-SLE = $0.142 \pm 0.031 \mu m^2/ms$, mean $D_{avg}(tCho)$ in healthy controls = $0.124 \pm 0.018 \mu m^2/ms$, $P = 0.044$). No significant difference was found in $D_{avg}(tNAA)$ values between patients with NP-SLE and healthy control subjects. No significant differences were observed in any metabolite D_{avg} between NP-SLE and SLE or between SLE and healthy control groups. SLEDAI-2K was used as a covariate when patients with SLE and those with NP-SLE were compared. When all SLE patients, with and without past CNS involvement, were grouped together and compared to healthy control subjects, $D_{avg}(tCr)$ and $D_{avg}(tCho)$ remained significantly higher in patients with SLE than in healthy control subjects after correcting for age [mean $D_{avg}(tCr)$ in all SLE patients = $0.190 \pm 0.034 \mu m^2/ms$, mean $D_{avg}(tCr)$ in healthy controls = $0.171 \pm 0.024 \mu m^2/ms$, $P = 0.060$ and mean $D_{avg}(tCho)$ in all SLE patients = $0.135 \pm 0.028 \mu m^2/ms$, mean $D_{avg}(tCho)$ in healthy controls = $0.124 \pm 0.018 \mu m^2/ms$, $P = 0.008$].

Average diffusion coefficients of all metabolites showed a link to disease activity in both the patients with SLE and those with NP-SLE. When all patients with SLE were pooled together, regardless of their neuropsychiatric status, SLEDAI-2K scores correlated positively with $D_{avg}(tNAA)$ ($r = 0.412$, $P = 0.033$), $D_{avg}(tCr)$ ($r = 0.405$, $P = 0.040$) and $D_{avg}(tCho)$ ($r = 0.480$, $P = 0.008$). Scatter plots of the SLEDAI-2K scores of all patients as a function of metabolite D_{avg} values are shown in **Figure 3**. When patients with SLE and those with NP-SLE categorized as SLE-active (SLEDAI-2 ≥ 8 , $n = 5$) were compared to healthy control subjects, statistically significant differences in $D_{avg}(tCr)$ were found [$D_{avg}(tCr)$ in SLE-active = $0.216 \pm 0.038 \mu m^2/ms$, $D_{avg}(tCr)$ in healthy controls = $0.171 \pm 0.024 \mu m^2/ms$, $P = 0.006$], as well as in $D_{avg}(tCho)$ [$D_{avg}(tCho)$ in SLE active = $0.154 \pm 0.037 \mu m^2/ms$, $D_{avg}(tCho)$ in healthy controls = $0.124 \pm 0.018 \mu m^2/ms$, $P = 0.006$] after correcting for age. No correlation was found between metabolite D_{avg} values and SLE duration or SDI scores.

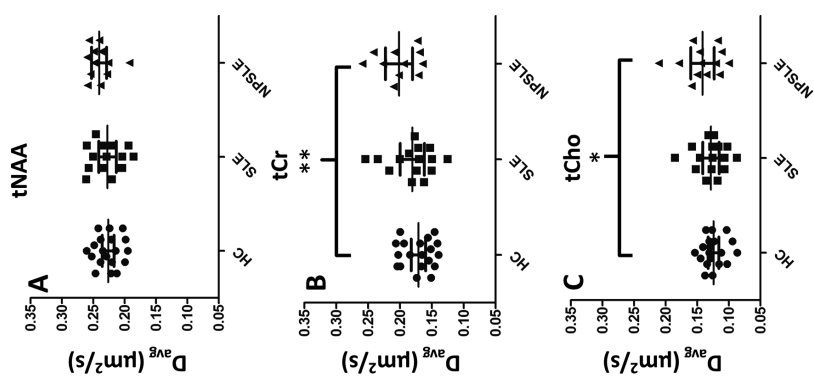


Figure 2. Metabolite Davg values for healthy controls subjects and patients with SLE and patients with NPSLE. Davg (tNAA), Davg (tCr) and Davg (tCho) data are shown in A, B and C, respectively. Statistically significant differences are shown as * $P < 0.05$ and ** $P < 0.01$. No significant differences were found between SLE and healthy control subjects, or between NPSLE and SLE in any of the metabolite Davg values. HC = healthy controls.

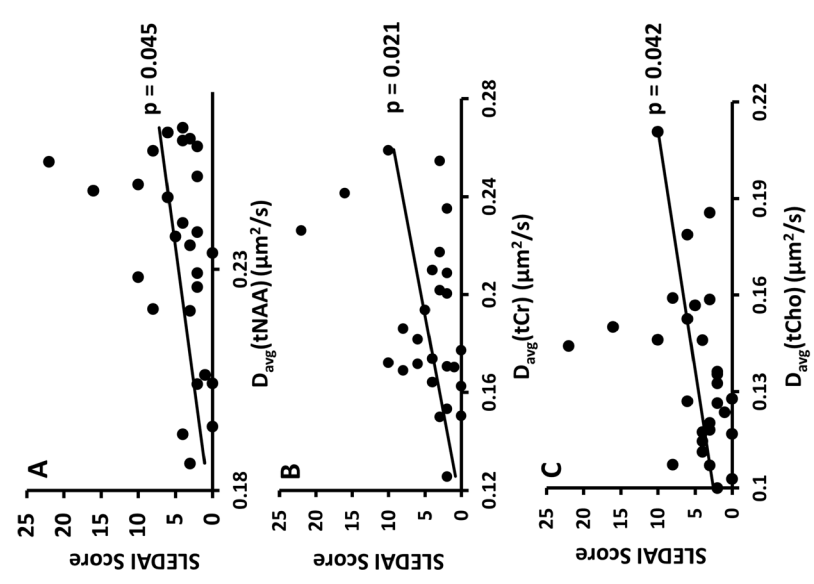


Figure 3. Correlation of metabolite Davg values with patient SLEDAI-2K scores. The resulting Spearman's rank correlation r and significance of the correlations are shown for Davg (tNAA) (A), Davg(tCr) (B) and Davg(tCho)

DTI and volumetric results

Significantly lower FA, higher MD and higher RD were found throughout white matter in the NP-SLE patient group compared to the healthy control and SLE groups, including the callosal region within the DW-MRS volume of interest ($P < 0.05$). **Figure 4** shows maps of statistically significant differences ($P < 0.05$) in DTI measures overlaid on the MNI152 T1weighted image, and the cumulative DW-MRS volume of interest (i.e. the sum of all the individual volumes of interest following transformation from the individual subject coordinates to the FA-MNI152 coordinates). The voxels with significantly higher mean and radial diffusivity values in the patients with NP-SLE compared to healthy controls are shown in blue and voxels with lower fractional anisotropy values in patients with NP-SLE compared to healthy controls are shown in red. No significant differences were found in any DTI measure between SLE patients (with and without past CNS involvement) and healthy control subjects. No significant differences were found in corpus callosum volumes or total brain volumes between patients with SLE or those with NP-SLE, and healthy control subjects.

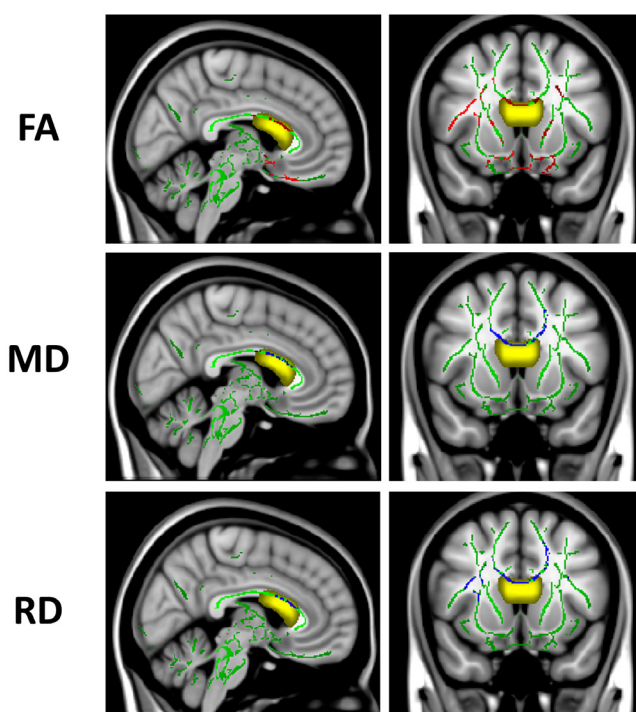


Figure 4. Tract-based spatial statistics results showing regions with statistically significant differences in DTI measures in the white matter skeleton of patients with NPSLE and healthy control subjects ($P < 0.05$). Maps are shown for one sagittal (left) and one coronal (right) slice in MNI152 space. The mean fractional anisotropy skeleton is shown in green, regions with higher values in the patients with NPSLE compared to healthy control subjects are shown in blue and regions with lower values in patients with NPSLE compared to healthy control are shown in red. Cumulative volume of interests chosen for DW-MRS of patients with NPSLE and healthy controls are shown in yellow.

FA = fractional anisotropy; MD = mean diffusivity; RD = radial diffusivity.

DISCUSSION

This is the first study to address cell-specific microstructural alterations in the brain of SLE patients with DW-MRS at ultrahigh field. This study focused on measuring the diffusion properties of two predominantly glial metabolites, tCr and tCho, and one exclusively axonal/neuronal metabolite, tNAA. The most salient finding in this study is the strong and consistent link between both $D_{avg}(tCr)$ and $D_{avg}(tCho)$ and disease state, with respect to disease activity and to past CNS involvement, suggesting glial involvement in the brain of these patients. Two potential pathological mechanisms that can explain the significantly higher tCr and tCho diffusivities found in NP-SLE patients are inflammation-mediated morphological changes in microglia and astrocytes, and intracellular edema, which would affect both glia and neurons/axons.(36-38)

Astrocytic and microglial reactivity in response to inflammation and/or ischemia are both highly consistent with an increase in intracellular diffusivity in glia. Reactivity-related cellular hypertrophy and thickening of the processes near the soma (especially in astrocytes) (39) would result in an increase of the intracellular space, and a decrease in molecular crowding and intracellular tortuosity, leading to increased diffusivity in the cytosol. The pathogenesis of NP-SLE is thought to involve various immune and inflammatory processes that can lead to neuronal injury and vasculopathy.(40) The inflammatory response to injury likely results in glial reactivity and cellular hypertrophy, especially in microglia and astrocytes.(37,38) Histopathological investigations of brains of NP-SLE patients, confirm the widespread presence of reactive microglia and astrocytes, as well as of lipid-laden macrophages among the heterogeneous pathological phenomena.(36) Furthermore, the correlation of *in vivo* MRS results with histological results from the same patients suggest: (a) an association between an increase in tCho concentrations and gliosis, vasculopathy and edema; (b) possible association of tCr with gliosis and reduced neuronal/axonal density; and (c) an association between lower tNAA concentrations and a decrease in neuronal/axonal density.(36)

The higher $D_{avg}(tNAA)$ values found in NP-SLE patients compared to HC may be attributed to changes in cytosolic viscosity in axons due either to neuronal/axonal damage or to cytotoxic edema, both of which are seen in the histopathology of brains of NP-SLE patients.(8) Higher $D_{avg}(tNAA)$ has also been observed in a study of patients with schizophrenia where it was hypothesized that inflammatory processes may play a role.(41) On the other hand, **lower** tNAA parallel diffusivity values were found in multiple sclerosis (MS) patients compared to HC in a study focused on myelin and axonal changes in the corpus callosum.(19) It is likely that the different behaviors in tNAA diffusivity seen in MS and in NP-SLE reflect different intra-axonal pathological mechanisms associated with these two diseases. Central to MS are demyelination and axonopathy.(42,43) As demyelination has no direct effect on diffusion in

the intra-axonal space, it has been hypothesized that in MS the decrease in tNAA axosolic diffusivity stemmed from axonal damage that included unusual patterns of neurofilament phosphorylation and packing compared to normal tissue, and a less organized axoskeleton and/or problems with axonal transport.(44) In contrast to findings in MS, histology of patients with NP-SLE have shown that cerebral edema occurs much more frequently than axonal/neuronal loss (8) and is thus more compatible with the increase in axosolic diffusivity, as evidenced by the increase in $D_{avg}(tNAA)$ observed in our study.

The high correlation in all SLE/NP-SLE patients between SLE disease activity, as quantified by the SLEDAI-2K score, and $D_{avg}(tCr)$ and $D_{avg}(tCho)$ suggests that the SLE-related peripheral inflammation and autoimmune response may have effect on the brain, independent of overt clinical CNS involvement in SLE. Additionally, significant correlation of $D_{avg}(tNAA)$ values with SLEDAI-2K scores suggests a permanent or continuous damage to axons correlated with high SLE activity. This is further corroborated by the finding that patients with higher disease activity (those we defined as SLE-active) have higher metabolite diffusivity levels and higher statistical significance in the difference in metabolite diffusivity compared to HC. A previous MRS study in NP-SLE/SLE has shown a significantly lower tNAA/tCr level in SLE patients with a high SLEDAI-2K score, and that the level of tNAA/tCr was renormalized in follow-up for patients who were no longer SLE-active, regardless of their NP status.(11) This finding suggests a pathological mechanism, attributed by the authors to neuronal dysfunction that affects both neurons and axons, the degree of which depends on SLE disease activity, but which is essentially reversible in nature. In our view, and based on our corroborative findings, we attribute this finding to intracellular/intraaxonal edema. It has been suggested that inflammation outside the brain can prime microglia and result in microglial activation for several weeks.(45) Our findings, as well as those described by Appenzeller et al.(46) support the view that systemic inflammation affects the brain in NP-SLE, and the underlying mechanism by which this occurs, e.g. potential disruption of the blood brain barrier in SLE (47), should be further investigated.

Metabolite apparent diffusion coefficient (ADC) values found in healthy controls in this study are similar to those reported in previous DW-MRS studies performed on a similar region of the corpus callosum at 7 tesla and 3 tesla.(21,29) A recently published robustness and reproducibility study of DW-MRS in the anterior body of the corpus callosum, aimed to provide guidelines for DW-MRS acquisition for clinical studies such as the one presented here. Using power calculations based on actual data it was estimated that in order to observe a 10% difference in D_{avg} values for tNAA, tCr and tCho in a case-control study, nine, four and twelve subjects, respectively, are sufficient.(29) In this current study we observed a 7% increase in $D_{avg}(tNAA)$, 19% increase in $D_{avg}(tCr)$ and 14% in $D_{avg}(tCho)$ in NP-SLE patients compared to HC, with thirteen and nineteen subjects per group, respectively. This

suggests that it is feasible to observe reliably the disease effect on metabolite D_{avg} reported here. Moreover, based on our power estimation, the number of subjects required to observe reliably a difference in $D_{avg}(tCho)$ is higher than that required for $D_{avg}(tNAA)$. This further supports the notion that our findings indicate a larger effect size for glial than for axonal involvement in SLE/NP-SLE. The DTI results are also consistent with previous studies on SLE and NP-SLE.(4,5,7) Significantly lower fractional anisotropy values in the anterior body of the corpus callosum of patients with NP-SLE compared to healthy controls were previously reported in a DTI study on NP-SLE where region of interest analysis was performed.(48) This study also included MRS measurements in normal appearing parietal white matter, and no differences in glial metabolite concentrations (tCr, tCho) between patients with NP-SLE and healthy controls were reported. Although changes in concentrations of glial metabolites have been linked to glial reactivity and neuroinflammation (49) this may suggest that the diffusion properties of glial metabolites are more sensitive to glial reactivity than their cellular concentration. Atrophy measurements did not show any significant callosal or global atrophy in the patient population, possibly stemming from a type II error due to the small size of the cohort.

There were several challenges and limitations to the study. The main (unmet) challenge of this study was to scan patients with active NP symptoms at the time of the scan, as well as to include more patients with high SLE disease activity. Low incidence of active NP-SLE was a factor, as well as significant potential discomfort to patients with clinically overt NP symptoms, preventing these patients from being involved in a research study that is not part of the routine clinical procedure. Another limitation of this study is the small number of patients per specific neuropsychiatric syndrome. This is a generally recognized problem in NP-SLE studies, mainly related to the low prevalence and the high heterogeneity of NP-SLE. Subsequently, definite conclusions concerning the relationship between DW-MRS findings and NP-SLE syndromes cannot be drawn. In our patient cohort, the relative low number of patients with antiphospholipid syndrome made it difficult to evaluate the effect of ischaemic/vascular changes on metabolite diffusion. As a consequence, we did not separately analyze changes in ischaemic and inflammatory patients with NP-SLE. Future studies will focus on separate evaluation of the effects of neuropsychiatric activity, ischaemic and inflammatory effects. The study would have benefitted from a separate MRS acquisition at short echo time, for an accurate evaluation of the metabolite concentrations in the same volume of interest, together with additional cell-specific metabolites such as glutamate, glutamine and myo-inositol.

In conclusion, the results presented in this study show for the first time that intracellular metabolite diffusion reflective of glial and neuronal/axonal involvement can be measured by DW-MRS in a complex autoimmune disease such as SLE/NP-SLE. This technique has great

potential for the study of the aetiology of disease-related changes in tissue microstructure of patients with SLE/NP-SLE. We believe that if incorporated in a comprehensive diagnostic scanning protocol together with existing microstructural MRI tools, such as DTI, MTI and susceptibility weighted imaging, DW-MRS can contribute to the diagnostic process of these patients and may help unravel underlying pathogenic mechanisms in this complex disease.

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part 3

**IMPROVING ATTRIBUTION OF
NEUROPSYCHIATRIC MANIFESTATIONS IN
SYSTEMIC LUPUS ERYTHEMATOSUS**

8

VALUE OF MULTIDISCIPLINARY REASSESSMENT IN ATTRIBUTION OF NEUROPSYCHIATRIC EVENTS TO SYSTEMIC LUPUS ERYTHEMATOSUS – PROSPECTIVE DATA FROM THE LEIDEN NP-SLE COHORT

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ABSTRACT

Objective: To determine the contribution of re-assessment in the attribution process of neuropsychiatric (NP) events to systemic lupus erythematosus (SLE) or other aetiologies in a large, prospective and multidisciplinary assessed NP-SLE cohort and to compare these results with other available attribution models for NP-events occurring in SLE.

Methods: Three-hundred and four consecutive SLE-patients presenting NP-events were evaluated. All subjects underwent standardized multidisciplinary medical, neuropsychological, laboratory and radiological examination in the inclusion and re-assessment dates. Diagnosis was always established by multidisciplinary consensus. The final diagnosis after re-assessment took also into account disease course and response to treatment. These data were compared with currently available attribution models for NP-events in SLE.

Results: A total of 463 NP-events were established. After re-assessment, attribution to SLE was discordant in 64 (13.8%) NP-events when compared with the first visit. We show that 14.5% of NP-events previously attributed to SLE reclassified as non-NP-SLE. In 86.4% of these patients immunosuppressive therapy was started after the first visit. When re-assessment and available attribution models were compared, NP-SLE cases overlapped considerably. Although specificity was high for all comparisons (0.81-0.95), an important variation in sensitivity (0.39-0.83) and agreement estimates (kappa = 0.29-0.68) was observed. The Italian algorithm showed the highest sensitivity and specificity (>0.80) and moderate agreement (0.59-0.64).

Conclusion: In clinical practice NP-events presenting in SLE are too often attributed to an immune-mediated origin. Multidisciplinary re-assessment avoids misclassification in NP-SLE. Multidisciplinary re-assessment is the reference standard in NP-events presenting in SLE and cannot be substituted by available attribution models.

Neuropsychiatric systemic lupus erythematosus (NP-SLE) is a term encompassing a broad and heterogeneous group of neuro-psychiatric (NP) symptoms as the consequence of the involvement of the nervous system due to systemic lupus erythematosus (SLE). The presentation of a NP-event in a patient with SLE represents a well-known diagnostic challenge.(1) So far, there is neither a gold standard nor a single complementary test that specifically discriminates between SLE-related and SLE-unrelated NP-events. Consequently, in clinical practice, NP-SLE is a diagnosis *per exclusionem*, requiring an extensive differential diagnosis orientated to the presenting NP-event.

The final attribution of NP-events to SLE is a crucial issue since it has important implications for management and prognosis. A scarce number of studies have rigorously analysed the attribution of NP-events to SLE. Hanly et al. reported that only one-third of all NP-events presented in a large SLE-cohort were directly attributable to SLE;(2,3) however, differences in the reported prevalence of NP-SLE vary widely (15-91%) due to different study designs among reports and more importantly due to the diverse interpretation of NP-SLE definitions. (4) For example, the nomenclature published by the American College of Rheumatology (ACR), the most comprehensive attempt to define NP manifestations in SLE patients, includes minor NP-events (e.g. headache); these are known to be nonspecific and to some degree investigator dependent.(5-9) In an attempt to assist physicians, some attribution models for NP-events occurring in SLE have been previously proposed. Despite the merits of these approaches,(2,3,6,10,11) in clinical practice, expert physician judgment based on clinical and complementary tests remains so far the most appropriate reference standard for NP-SLE diagnosis.(12)

In SLE patients presenting for the first time with a NP-event, recognizing an SLE-related origin can be difficult, and sometimes the diagnosis of NP-SLE will be presumptive. Re-assessment of NP-SLE patients may be thus of paramount importance in the attribution process. At a follow-up visit, the clinical course and the response to therapy harbour crucial information that will help in the attribution of NP-events to SLE or other aetiology.(13-15) To the best of our knowledge, the value of re-assessment of NP-events in SLE patients has never been addressed before and it is unknown how the evaluation over time may provide insight into this complex and important aspect of SLE.

The aim of the present study is therefore (a) to determine the contribution of re-assessment in the attribution of NP-events to SLE or to other aetiologies in a large, prospective and multidisciplinary assessed SLE-cohort. Assuming that the course of disease leads to a putative reference standard, this also allows us to (b) assess the accuracy of the first visit and compare these results with all available attribution models for NP-events occurring in SLE.

METHODS

Subjects

Our study group comprised patients with SLE and NP-events from the Leiden NP-SLE-cohort. Our institution, the Leiden University Medical Centre (The Netherlands), is a national tertiary referral centre for NP-SLE where patients are evaluated in a multidisciplinary, standardized and prospective manner. Between September 2007 and March 2016, a total of 304 consecutive patients who were suspected by a referral doctor of having NP-SLE were evaluated. The local medical ethic committee approved the study and all patients signed informed consent.

Multidisciplinary assessment

All subjects included in our NP-SLE-cohort were admitted for a 1-day period and underwent standardized multidisciplinary assessment. In a small number of cases, this evaluation took place during a regular clinical admission in our centre. During the first visit all patients were assessed by specialists in rheumatology, neurology, psychiatry and vascular medicine. In addition, neuropsychological testing, extensive laboratory tests and a 3-Tesla magnetic resonance imaging (MRI) of the brain were routinely performed. Additional cerebrospinal fluid analysis, electromyogram, electroencephalogram, evoked potentials, MRI of the spine or MR-angiography were performed when indicated. For a detailed description of the evaluations included in the clinical assessment, neuropsychological test battery, laboratory tests and MRI-scanning protocol, see reference (15). Among the socio-demographic and clinical variables obtained were age, gender, disease duration and activity, lag time between SLE diagnosis and NP-event presentation and therapies used before and after the first visit. All patients were classified according to the ACR 1982 revised criteria for SLE. [16,17] SLE disease activity was calculated with the Systemic Lupus Erythematosus Disease Activity Index 2000.(18,19)

Consensus meeting

The final attribution of NP-events to SLE or other aetiologies was made by multidisciplinary consensus after the clinical, serological and neuroimaging assessments and evaluation of neuropsychological status and competing co-morbidities. All medical specialists met in a 2-weekly scheduled meeting to discuss the patients. In acute cases where a prompt therapeutic decision was needed an extra meeting was planned. As described by Zirkzee et al. (15), during the consensus meeting the following aspects were taken into account to determine the origin of NP-events: objective confirmation of symptoms (assessed to standard of care of the appropriate medical specialty); exclusion of other aetiology explaining these symptoms; NP-event possibly explained by SLE. All identified aetiologies for NP-events

and the NP-SLE definitions were defined, but not restricted, according to those alternative diagnosis and the NP-SLE definitions included in the 1999 ACR nomenclature.(5) Since a patient may present several NP-events due to different aetiologies, we have chosen to analyse every NP-event individually instead of per patient. We also identified the so-called minor NP-events as defined by Ainiala et al., including headache, anxiety, mild depression, mild cognitive impairment and polyneuropathy without electrophysiological confirmation.(7,9) Each NP-event was attributed to one of the following groups: NP-SLE or NP-events directly related to SLE, undefined NP-SLE when we were not able to neither find another aetiology nor clearly associate the symptoms with SLE, and non-NP-SLE or NP-events better explained by other aetiology. Furthermore, non-NP-SLE events were divided into the next subgroups: due to primary NP disease, due to medication or drugs, due to a complication of SLE (e.g. strokes following Libman-Sacks endocarditis) and due to other concomitant disease. After the attribution process, the group of specialists made a therapeutic decision per NP-event: initiate immunosuppression therapy, secondary prevention, optimize symptomatic therapy, start psychotherapy or stop a specific therapy when the NP-event was thought to be medication-related. NP-events classified as undefined NP-SLE where treated with intensive symptomatic therapy while a subset of NP-events classified as NP-SLE not responding to a previous appropriate symptomatic therapy received a trial with immunosuppressive therapy.(10,13,15) All patients included in this group received glucocorticoids (0.5-1 mg/kg/d) and concomitant azathioprine or mycophenolate mofetil. We decided the date of the re-assessment depending on the NP-event, severity and therapy established. The referral doctors closely followed all patients until the follow-up visit.

Follow-up visit

Re-assessment of patients took place three to 18 months after the first visit. Several NP-events included in the group non-NP-SLE were not reassessed when they were clearly explained by other disease (e.g. cerebral tumour). Patients were again admitted for a 1-day period and underwent the same multidisciplinary assessment as during the first visit. All patients were re-assessed by the same specialists. Moreover, neuropsychological battery testing, laboratory and radiological examination using the same protocol were performed and compared with previous tests. For a further description of multidisciplinary assessment and performed tests at this point see reference (15). Two weeks after re-assessment a consensus meeting took place and the following factors were taken into account for every NP-event: evolution over time and evaluation of improvement or worsening of previous evaluated NP-events; onset of new NP-events or other symptoms related and non-related to SLE that may explain or contribute to new and previous NP-events. NP-events better explained by other aetiologies were divided into the same groups as described after the first visit.

Other attribution models

Three different models for the attribution of NP-events to SLE have been proposed. The Systemic Lupus International Collaborating Clinics (SLICC) group developed two different models with different stringency (model A more stringent and B less stringent) taking into account the temporal relationship between the NP-event and SLE diagnosis, the presence of exclusions or associations described in the 1999 ACR nomenclature and whether the NP-event was one of the minor NP-events described by Ainsworth et al. (2,5,7,9). Attribution of a NP-event to SLE by the SLICC attribution model A (SLICC-A) included: NP-event onset from 6 months prior to 15 months after SLE diagnosis, was not a minor NP-event and exclusions or associations as described in the ACR nomenclature were not present; and in the case of SLICC attribution model B (SLICC-B): NP-event onset within 10 years from SLE diagnosis, was not a minor NP-event and exclusions as described in the ACR nomenclature were not present.

Recently, the Italian study group on NP-SLE proposed an algorithm for the attribution of NP-events to SLE based on a probability score.(10) This model addresses: temporal relationship of NP-events to SLE diagnosis; identification of minor NP-events; recognition of confounding factors as described in the ACR nomenclature for NP-SLE; and favouring factors including the specific SLE-related risk factor derived from the European League Against Rheumatism recommendations on NP-SLE and other further information considered of importance for the group.(10,20) The authors proposed two cut-off points in a scale from 0 to 10 points. NP-events with a score of < 3 were considered as due to non-SLE causes, between 3 and 6 as undefined and NP-events with a score of 7 or more as due to SLE.(10)

Statistical analysis

SPSS version 20.0 for Windows (IBM SPSS statistics, Chicago, IL, USA) was used for analysis. Differences between qualitative variables were assessed using χ^2 test. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for re-assessment against the other attribution models and first visit were determined. We assumed that the course of every NP-event leads to a putative reference and subsequently we used the final attribution after multidisciplinary re-assessment as gold standard. We also analysed the performance of the attribution models when compared with the first visit. In the case of the first visit and the Italian algorithm we used dichotomous outcomes, including undefined and non-NP-SLE in the same group. Agreement between re-assessment, first visit and other attribution models was calculated with the use of Cohen's kappa measures of agreement.

RESULTS

Patient’s characteristics

A total of 304 SLE patients were evaluated. The median age of all patients at the time of the study was 42.5 years (interquartile range [IQR] 33-50) and 89.7% were female. The median duration of SLE was 4.6 years (IQR 1.2-13.2); In 11 patients we were not able to establish a NP diagnosis at the first visit. The rest of the 293 patients were diagnosed with at least one NP-event; a total of 463 NP-events were established. **Table 1** shows the attribution of all 463 NP-events after the first visit. The median number of NP-events presented in all NP-SLE patients was 1 (IQR 1-2; range 1–5). NP-SLE patients also presented 26 concomitant NP-events non-attributed to SLE and 9 NP-events classified as undefined. NP-events attributed to SLE developed after a median of 1.9 years (IQR 0.2-11.6). A total of 65 (42.8%) NP-events attributed to SLE presented in the first year after SLE diagnosis. In 37 of these 65 NP-events, NP-SLE was diagnosed at the same time as SLE.

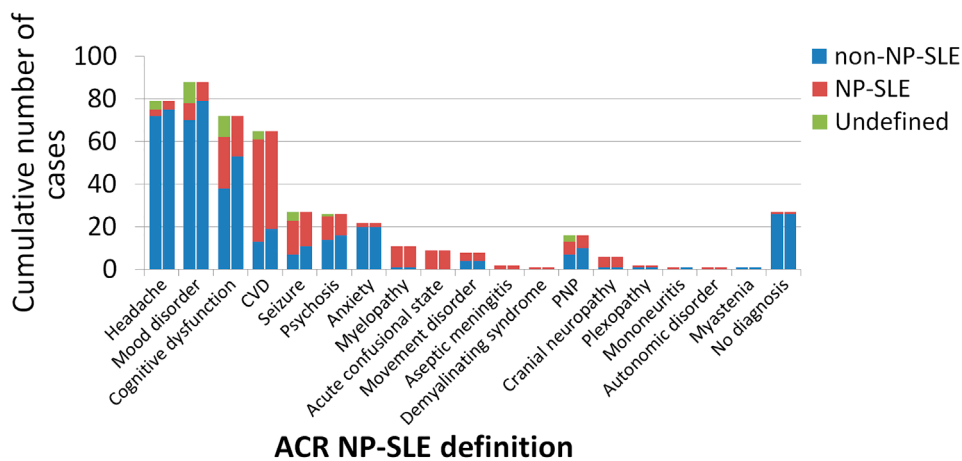


Figure 1. Summary of NP-events (n = 463) after the clinical judgment at first visit and re-assessment

Figure 1 shows all 463 NP-events distributed according to attribution at first visit and re-assessment. Besides Guillain-Barre syndrome and myasthenia gravis, all ACR definitions were represented in our cohort. Among all the NP-events, a total of 224 were identified as minor NP-events.(7,9) In our cohort, a low number of NP-events diagnosed as headache (4/79; 5.1%), mood disorder (9/88; 10.2%), cognitive dysfunction (19/72; 26.3%) or anxiety (2/22; 9.1%) were attributed to SLE.

Table 1. Attribution of 463 neuropsychiatric events presenting in 304 SLE patients from the Leiden NPSLE-cohort at first visit and after re-assessment

			Attribution re-assessment		
			Non-NP-SLE	NP-SLE	Total
Attribution first visit	Non-NP-SLE	Count	269	6	275
		% total	58.1	1.3	59.4
	NP-SLE	Count	22	130	152
		% total	4.8	28.1	32.8
	Undefined	Count	27	9	36
		% total	5.8	1.9	7.8
	Total	Count	318	145	463
		% total	68.7	31.3	100.0

NP-SLE: neuropsychiatric systemic lupus erythematosus

Comparison between first visit and re-assessment

The final attribution of NP-events to SLE or other aetiologies after re-assessment was analysed (**Table 1**). Most of the patients were re-assessed 6 months (IQR 4-12) after the first visit. Of the 152 NP-events attributed in the first to SLE, a total of 22 (14.5%) were reclassified to the non-NP-SLE group. Of these 22 NP-events, in 19 (86.4%) an immune-mediated origin of the symptoms was suspected at first visit and immunosuppressive therapy was started. A further description of NP-events re-included in the NP-SLE group after re-assessment is available in the **Supplementary Table 1**. On the other hand, of the 275 NP-events previously related to non-SLE aetiologies, only 6 NP-events (2.2%) were reclassified into the NP-SLE group (see **Figure 2** for flow chart of NP-events). In total, there were 64 (13.8%) discordant NP-events when the first visit and re-assessment were compared. Agreement between the first visit and re-assessment was very good ($\kappa = 0.82$).

After re-assessment, among the 318 NP-events non-attributed to SLE, a total of 217 were better explained by other primary NP aetiology, 39 NP-events were attributed to medication or drugs, 26 due to a concomitant non-NP-disease, 10 were related to a SLE-complication and in 26 NP-events we were not able to establish a diagnosis included in the ACR nomenclature. A summary of all alternative diagnosis in the non-NP-SLE group is available in the **Supplementary Table 2**.

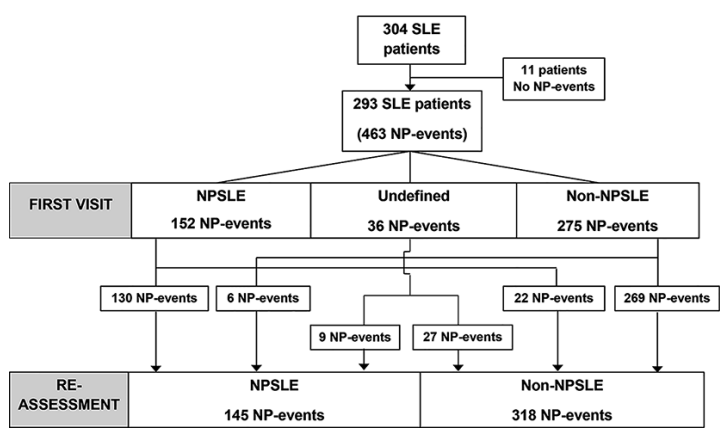


Figure 2. Flow chart of NP-events presented in the Leiden NPSLE-cohort

A total of 36 NP-events were categorized after the first visit as undefined NP-SLE and subsequently treated with intensive symptomatic therapy. After re-assessment these NP-events reclassified into NP-SLE (9 NP-events) and non-NP-SLE (27 NP-events). Furthermore, a total of 28 NP-events attributed to SLE not responding to a previous appropriate symptomatic therapy received a trial of immunosuppressive therapy after the first visit. After re-assessment 19 NP-events had a favourable response and were definitely attributed to SLE while 9 NP-events did not respond and were thought to be non-SLE related.

Table 2. NP-events attributed to SLE at the first visit and re-assessment and according to different attribution models

	Non-NP-SLE	First visit	Re-assessment	SLICC-A	SLICC-B	Italian algorithm
Non-NP-SLE	243	311	318	407	324	287
First visit	311	152*	130	50	97	126
Re-assessment	318	130	145*	42	90	117
SLICC-A	407	50	42	56*	56	52
SLICC-B	324	97	90	56	139*	124
Italian algorithm	287	126	117	52	124	176*

* Number of NP-events attributed to SLE. NP-SLE: neuropsychiatric systemic lupus erythematosus; SLICC: Systemic lupus international collaborating clinics.

Comparison and agreement with other attribution models

The number of NP-events attributed to SLE varied considerably when the different attribution models were used (**Table 2**). Of all NP-events, a total of 38 (8.2% of the total NP-events) and

243 were respectively attributed to SLE or to other aetiology by all the different attribution approaches. When compared with the re-assessment, specificity was high for all models (0.81-0.95) while sensitivity changed importantly depending on the models analysed (0.29-0.81).

Table 3. Accuracy indexes, predictive values and Cohen´s kappa measures of agreement between NP-SLE diagnosis after re-assessment, first visit and different attribution models

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Kappa (95% CI)
Re-assessment/First visit*	0.89 (0.83-0.94)	0.93 (0.89-0.96)	0.85 (0.79-0.91)	0.95 (0.92-0.97)	0.82 (0.76-0.87)
Re-assessment/SLICC-A	0.29 (0.22-0.37)	0.95 (0.93-0.98)	0.75 (0.62-0.86)	0.75 (0.70-0.79)	0.29 (0.21-0.38)
Re-assessment/SLICC-B	0.62 (0.54-0.70)	0.85 (0.80-0.88)	0.65 (0.56-0.73)	0.83 (0.78-0.87)	0.47 (0.38-056)
Re-assessment/Italian algorithm	0.81 (0.73-0.87)	0.81 (0.76-0.86)	0.66 (0.59-0.73)	0.90 (0.86-0.93)	0.59 (0.51-0.66)
First visit/SLICC-A	0.33 (0.25-0.41)	0.98 (0.96-0.99)	0.89 (0.78-0.96)	0.75 (0.70-0.79)	0.37 (0.28-0.45)
First visit/SLICC-B	0.64 (0.56-0.71)	0.86 (0.82-0.90)	0.70 (0.61-0.77)	0.83 (0.78-0.87)	0.51 (0.43-0.60)
First visit/Italian algorithm	0.83 (0.76-0.88)	0.84 (0.79-0.88)	0.72 (0.64-0.78)	0.91 (0.87-0.94)	0.64 (0.57-0.71)

*First group in the first column indicates the group used as reference standard. NP-SLE: neuropsychiatric systemic lupus erythematosus; NPV: negative predictive value; PPV: positive predictive value; SLICC: Systemic lupus international collaborating clinics

Comparable results were found when first visit was used as reference and compared with all attribution models. PPV was low when attribution models were compared with re-assessment (0.65-0.75) while NPV was higher (0.75-0.90). A very low sensitivity was specially marked for SLICC-A due to the small number of NP-events attributed to SLE. When compared with first visit and re-assessment, the Italian model showed a reasonably high sensitivity (0.83 and 0.81, respectively), specificity (0.84 and 0.81, respectively) and NPV (0.91 and 0.90, respectively). The agreement was good when the Italian algorithm was compared with the first visit and re-assessment (kappa = 0.64 and 0.59, respectively). Among the rest of the comparisons, the agreement was poor to moderate (**Table 3**).

DISCUSSION

To the best of our knowledge, this is the first study where a large cohort of SLE patients presenting NP-events is evaluated in a prospective manner by a multidisciplinary team. We have been able to characterize 463 NP-events occurring in 293 SLE patients using this multidisciplinary assessment and to follow the evolution of NP-events over time.

We show that re-assessment of NP symptoms in SLE reclassifies a total of 13.8% NP-events. Interestingly, of all NP-events first misdiagnosed as NP-SLE, 86.4% were attributed to an immune-mediated origin and received immunosuppression. Our data suggests that, in clinical practice, over-diagnosis related with an immune-mediated origin is more common than under-diagnosis in NP-SLE. This demonstrates the complexity and the difficulties found in clinical practice to reach an accurate diagnosis at the first visit. There was an acceptable interrater agreement between the two visits, which may have different interpretations. Diagnosis during first visit seems accurate since we were able to identify the majority of real non-NP-SLE and NP-SLE events. However, we think that having 64 (13.8%) NP-events with an incorrect attribution after the first visit is a serious problem and stresses the fact that new and more reliable tests for NP-SLE are urgently needed.⁽²¹⁾ Until those tests are available, it will be very likely that we over-diagnose NP-SLE in SLE patients presenting with NP-events. Therefore, we believe that multidisciplinary re-assessment is so far the most accurate approach to attribute NP-events to SLE. Furthermore, this increasing of diagnostic accuracy in NP-SLE may have important consequences not only in individual patients but also for studies of physiopathology mechanisms and therapy trials.

We have analysed the role of the response to therapy in the attribution of NP-SLE. After re-assessment, and subsequently after receiving intensive symptomatic therapy, we were able to establish a final diagnosis for all NP-events categorized as undefined NP-SLE in the first visit. Furthermore, in 14.6% of the NP-events attributed to SLE after the first visit, the final diagnosis could only be confirmed after evaluating the response to a trial of immunosuppressive therapy. Our findings demonstrate the importance of monitoring the evolution of NP-events and its response to specific therapeutic interventions in the attribution process of NP-events to SLE.

We have been able to describe and divide into four different groups the alternative aetiologies explaining the NP-events other than SLE. The diagnosis of other primary NP disease accounts for 68.2%. Among them a total of 88.9% NP-events correspond to minor NP-events.^(7,9) The presence of these minor NP-events is known to substantially influence the prevalence of NP-SLE.⁽⁴⁾ Although minor NP-events were considered the most prevalent NP-SLE events in the past, definitions have evolved over time as has medical knowledge; in the last years there

is a tendency to consider that in most of these minor NP-events there exists not a subjacent immune-mediated origin.(8,9,13,22,23) Our data confirm the low rate of SLE-attribution for these minor NP-events and points out the importance of an exhaustive differential diagnosis and the need for a more stringent approach to attribute NP-events to SLE.

We have compared different accepted attribution models for NP-events presented in SLE patients. We demonstrated the important variation in frequency and agreement estimates and how NP-SLE diagnosis overlaps considerably. The high specificity and NPV and on the other hand the wide variability in sensitivity points out that all attribution models recognize reasonably well non-NP-SLE events, while the recognition of NP-events associated to SLE is more problematic. These data show that available attribution models are more useful for the exclusion of NP-SLE than for its recognition. The high specificity and the low sensitivity and agreement of SLICC-A respond to the strict requirements of this model. Two factors have a substantial influence in these results: the stringent enrolment window; and the exclusion of a patient as NP-SLE when recognizing non-SLE variables (e.g. coexistent conditions or drugs) as having contributed in part to the NP-event, the so-called associations.(2) The SLICC-B model, a less strict model, showed a moderate sensitivity and agreement. Of note, the Italian algorithm showed an acceptable sensitivity and specificity, a high NPV and good agreement when compared with the first visit and re-assessment. Recently, a retrospective study has shown similar results.(24) Our data shows that this method performs reasonably well with both the first visit and re-assessment and may be a reliable tool for research matters; however, as already acknowledge by the authors, we believe that this algorithm should not be used as a substitute of clinical judgment.(10)

There are limitations in this study. Our study shares with other studies a heterogeneous and small frequency of certain NP-SLE syndromes. This avoids drawing firm conclusions about how re-assessment may affect specific NP-SLE syndromes; however, our cohort is formed by patients referred from all over the country and covers a large part of the NP-SLE spectrum, which may lead to a more real-life setting, thereby diminishing confounding and allowing for the generalizability of our results. Among many other factors, the attribution at first visit was taken into account to reach a final decision at re-assessment, which may have led to incorporation bias and subsequently to an overestimation of test accuracy for the first visit when compared with other attribution models. Nevertheless, our study is based in a clinical setting. At re-assessment, it is imperative to know the attribution at first visit to know if a certain NP-event has been misclassified. Although unlikely, the possibility that at re-assessment a NP-event improved due to the waxing and waning nature of SLE rather than to a good response to anti-inflammatory therapies cannot be excluded. Another limitation may be the variance in follow-up intervals. Re-assessment was scheduled after 6-18 months for most of the patients. These intervals were chosen because we are used to give courses of

anti-inflammatory therapy for at least six months and sometimes extend these to 18 months (e.g. cyclophosphamide). Furthermore six months seems enough time to evaluate the effect of symptomatic therapies (e.g. anti-depressive therapy). Although patients were closely followed by referral doctors, we do not know how additional information from in between visits could have yield valuable information about the evolution of NP-events.

In summary, this is the first study evaluating the effect of re-assessment of NP-events presented in a large prospective SLE-cohort. Our data shows that multidisciplinary clinical assessment is useful to attribute NP-events to SLE or to other aetiologies; however NP-SLE may be over-diagnosed and too often attributed to an immune mediated origin. Re-assessment helps to avoid misclassification in attribution of these NP-events to SLE. Evaluating the response to empiric therapies in certain subsets of patients is a key-element at this point. Existing attribution models for NP-SLE cannot replace clinical judgment; however the Italian algorithm is relatively accurate and a potential tool to be used for research matters. Until we have more reliable serological or radiological biomarkers, we recommend multidisciplinary re-assessment as the standard of care in SLE patients presenting NP-events.

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SUPPLEMENTARY MATERIAL

Supplementary table 1. Causes for inclusion in the NP-SLE group after re-assessment

First diagnosis	Cause for diagnosis NP-SLE
Patients first diagnosed as non-NP-SLE (n = 6)	
Headache – tension headache	CVD – sinus thrombosis
Headache – Migraine without aura	Lupus headache
Adjustment disorder with depressive mood	Mood disorder with depressive features – SLE related
Mood disorder with depressive features – Primary mood disorder	Major depressive like episode – SLE related
Mild cognitive complaints thought to be subjective	Severe cognitive dysfunction – SLE related
Patients first diagnosed as undefined (n = 9)	
Seizure – absences and tonic-clonic seizures	Not responding to maximal dosing of several antiepileptic therapies and only responding after high doses corticosteroids and azathioprine
Headache – doubt between migraine and lupus headache in an active SLE	Recalcitrant headache non responding to maximal dosing of several symptomatic therapies and only responding after corticosteroids
Psychosis – doubt between corticosteroid related psychosis and SLE-psychosis	Psychosis worsening after stopping corticosteroid and including maximal dosing antipsychotic therapy and only responding after high doses corticosteroids and cyclophosphamide
Mood disorder with depressive features – doubt between corticosteroid side effect and SLE related mood disorder	Mood disorder worsening together with SLE activity after stopping corticosteroid not responding to several optimal anti-depressive drugs and only responding after high doses corticosteroids and azathioprine
Mood disorder – major depressive-like episode – doubt between primary mood disorder and SLE related mood disorder	Only response to corticosteroids and azathioprine after several symptomatic therapies
Cognitive dysfunction – doubt between due to past ischemia or to disease activity	No response to psychotherapy. Only response after adjustment of immunosuppressant and mild doses corticosteroids
Headache and CVD – active SLE with headache and paresis of the left arm with a normal MRI (clinico-radiological paradox)	Intractable headache after maximal dosing of several symptomatic therapies. Both headache and paresis only responding after high doses corticosteroids and azathioprine

CVD: cerebrovascular disease; MRI: magnetic resonance imaging; SLE: systemic lupus erythematosus

Supplementary table 2. Alternative diagnosis in the non-NP-SLE group after re-assessment of all neuropsychiatric events (n = 318)

Alternative diagnosis [†]	Number of NP events [*]
Primary NP etiology (n =217)	
Cerebrovascular disease	
Oligodendroglioma	1 (1)
Cerebral small vessel disease related to cerebral amyloid angiopathy	1
Cognitive dysfunction	
Non-related to SLE	27 (4)
Fronto-temporal dementia	1
Fahr disease	1
Cerebral amyloid angiopathy	1
Related to Parkinson	1
Mood disorder	
Mood disorder with depressive features	32 (2)
Major depressive-like episode	24
Depressive disorder NOS	6 (1)
Bipolar disorder	5
Mood disorder with maniac features	3
Mood disorder with mixed features	2 (1)
Dysthymic disorder	2
Post-stroke depression	1
Headache	
Migraine without aura	18
Migraine with aura	15
Tension-type headache	13
Headache non-specific	6
Headache attributed to sinus venous thrombosis	4
Headache attributed to ischemic stroke	2 (1)
Headache attributed to intracranial neoplasm	2
Cluster headache	2
Headache related to unruptured vascular malformation	1
Movement disorder	
Dystonia	2
Parkinson disease	1
Psychosis	
Schizophrenia	7 (2)
Schizoaffective disorder	1
Seizures	
Focal seizure	4
Generalized seizure	3 (1)
Unknown seizure	1
Anxiety	
Generalized anxiety disorder	9
Panic disorder with/without agoraphobia	7
Posttraumatic stress disorder	2
Specific phobia	1
Polyneuropathy	
Light polyneuropathy not confirmed in electromyogram	4 (1)
Small fiber neuropathy due to other cause	1
Mononeuritis	
Somatoform disorder (functional disorder)	1 (1)
Cranial neuropathy	
Cranial neuropathy abducens nerve	1
Plexopathy	
Damage due to compression L4-L5	1
Medication or drug related (n = 39)	
Cerebrovascular disease	
Vertigo and absences due to benzodiazepines	1
Cognitive dysfunction	
Related to prednisone	6 (1)
Drug abuse – cannabis substance dependence	2
Related to ibuprofen	1
Related to Risperdal	1
Progressive multifocal leukoencephalopathy due to mycophenolate mofetil	1

Mood disorder	
Substance induced mood disorder – cannabis and alcohol	2
Substance-induced psychotic disorder – prednisone	2
Headache	
Medication overuse headache	11 (1)
Headache induced by acute substance or exposure	2
Anxiety	
Substance induced anxiety disorder – alcohol	1
Psychosis	
Drug-induced psychotic disorder – prednisone	5
Drug-induced psychotic disorder – cannabis	2
Drug-induced psychotic disorder - hydroxychloroquine	1
Polyneuropathy	
Side effects acetazolamide	1
Concomitant extra-cranial disease (n = 26)	
Cerebrovascular disease	
Stroke related to multiple cardiovascular risks	3 (1)
TIA related to multiple cardiovascular risks	2 (1)
Age and hypertension-related cerebral small vessel disease	2
Hypertensive encephalopathy	2
Embolism due to atrial fibrillation	2
Vasovagal syncope	2
Embolism due to auricular flutter	1
Carotid dissection	1
Cognitive dysfunction	
Paraneoplastic breast-carcinoma	1
Nocardia infection	1 (1)
Seizures	
Metabolic disorder	1
Vasovagal syncope	1
Seizure post-stroke after auricular flutter	1
Myelopathy	
Post-herpetic myelitis	1
Myasthenia gravis	
Lambert-Eaton	1
Polyneuropathy	
Epidural lipomatosis	1 (1)
Hypothyroidism	1
Diabetic neuropathy	1
Sjögren syndrome	1
Complication SLE (n = 10)	
Cerebrovascular disease	
Libman-Sacks endocarditis	1
Cognitive dysfunction	
Complication ischemia	7 (1)
Libman-Sacks endocarditis	1
Movement disorder	
Myoclonus-dystonia related to severe ischemia	1
Other diagnosis (n = 26)	
Adjustment disorder with anxiety (maladaptive to stress due to having SLE)	6
Dissociative disorder	4
Somatoform disorder (functional disorder)	4
Adjustment disorder with depressed mood	3
Personality disorder – Obsessive-compulsive	2
Sleep disorder - Nightmare disorder	2
Adult antisocial behavior	1
Personality disorder – Borderline	1
Eating disorder - Anorexia nervosa	1
Attention-Deficit and Disruptive Behavior disorders (ADHD)	1
Birdshot disease	1

ADHD: attention deficit hyperactivity disorder; NOS: not otherwise specified; NP: neuropsychiatric;

SLE: systemic lupus erythematosus; TIA: transient ischemic attack

(*) Numbers in parenthesis indicate the patients previously diagnosed as NP-SLE.

(†) Psychiatric pathology was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV). [1] Headaches were classified according to the International Classification of Headache Disorders ICHD. [2]

9

OUTCOMES OF NEUROPSYCHIATRIC EVENTS IN SYSTEMIC LUPUS ERYTHEMATOSUS BASED ON CLINICAL PHENOTYPES – PROSPECTIVE DATA FROM THE LEIDEN NP-SLE COHORT

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ABSTRACT

Objective: To assess whether clinical and patient's reported outcomes are associated with a different pathophysiologic origin of neuropsychiatric (NP) events presenting in systemic lupus erythematosus (SLE).

Methods: A total of 232 NP-events presenting in 131 SLE-patients were included. NP-SLE diagnosis was established per event by multidisciplinary evaluation. All NP-events were divided according to a suspected underlying pathophysiological process into one of the next: non-NP-SLE related, inflammatory and ischemic NP-SLE. The clinical outcome of all NP-events was determined by a physician-completed four-point-Likert scale. Health-related quality of life was measured with the subscales of the patient-generated Short Form 36 (SF-36) health survey questionnaire. The change between scores at paired visits of all domain scores, mental component summary (SF-36 MCS) and physical component summary (SF-36 PCS) scores were retrospectively calculated and used as patient reported outcome. The association among these outcomes and the different origin of NP-events was obtained using multiple logistic regression analysis.

Results: The clinical status of 26.8% non-NP-SLE events, 15.8% ischemic NP-SLE and 51.6% inflammatory NP-SLE improved after re-assessment. Almost all SF-36 domains had a positive change at re-assessment in all groups independently of the origin of NP-events. NP-SLE ($B = 0.502$; $p < 0.001$) and especially inflammatory NP-SLE ($B = 0.827$; $p < 0.001$) had better clinical outcome being change in disease activity the only important predictor. The change in SF-36 MCS was also independently associated with NP-SLE ($B = 5.783$; $p < 0.05$) and inflammatory-NP-SLE ($B = 11.133$; $p < 0.001$). Disease duration and change in disease activity were the only predictors in both cases. The change in SF-36 PCS was only negatively associated with age.

Conclusion: Inflammatory NP-SLE events have better clinical outcome and a meaningful improvement in SF-36 MCS than ischemic NP-SLE or non-NP-SLE.

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease that has protean manifestations.(1) Nervous system involvement in SLE leads to a heterogeneous group of neurological and psychiatric symptoms (Neuropsychiatric systemic lupus erythematosus). Any of these neuropsychiatric (NP)-events can be directly attributed to SLE (NP-SLE) or to an alternative aetiology (non-NP-SLE). Although NP-SLE pathogenesis is incompletely understood, two underlying mechanisms are recognized: a) Inflammatory NP-SLE: associated with dysfunction due to pathogenic antibodies with a disrupted blood-brain barrier, and b) ischemic NP-SLE: associated with focal neurological deficits due to the interruption of the blood-flow in a specific region of the brain.(2,3) In order to guide therapeutic decisions in clinical practice, we have previously proposed a pathophysiological clustering of NP-SLE patients based in these two mechanisms; therapy is thus directed to inflammation with immunosuppressive therapy or to ischemia/thrombosis with anticoagulants and antiaggregants.(2)

The clinical outcome of NP-events presenting in SLE has been scarcely studied. A 2-year follow-up study of 32 hospitalized NP-SLE patients showed an improvement and stabilization of symptoms in 69% and 19%, respectively.(4) Some authors have not found a difference in outcome when the aetiology of NP-events (NP-SLE vs non-NP-SLE) was analysed.(5) Two previous investigations explored the short and long-term outcome of NP-events, regardless its aetiology, presenting in the large inception Systemic Lupus International Collaborating Clinics (SLICC)-cohort. Both analysis found that the outcome of NP-SLE-events were more favourable than in non-NP-SLE-events.(6,7)

The occurrence of NP-events in SLE patients, independently of its aetiology, has been associated with a considerable comorbidity resulting in a marked adverse repercussion on health related quality of life (HRQoL).(6) Among all the available tools for measuring HRQoL, the 36-item Short-Form Health Survey (SF-36) is a valid and reliable tool to identify the effect of SLE in the physical, mental and social domains of these patients.(8-10) Previous research has shown how SF-36 is associated with the clinical outcome of NP-events in SLE patients, especially the domains concerning self-report mental health where the improvement of disease activity may play an important role.(11)

So far, the clinical outcome and HRQoL of NP-events in SLE have never been investigated in a large multidisciplinary assessed NP-SLE-cohort. Moreover, it is unknown how a certain underlying pathophysiological mechanism of NP-events presenting in SLE may impact clinical outcome and SF-36 domains change over time. Inflammatory NP-SLE may be thought to have a better outcome after immunosuppressive therapy is given and subsequently the origin of the NP-event eradicated while a smaller improvement may be expected in ischemic NP-SLE after receiving secondary prevention.

Our current work aims to (a) assess clinical outcome and change in HRQoL measured by SF-36 on a multidisciplinary assessed and prospectively followed cohort of SLE patients with NP-events either related and non-related to SLE, (b) investigate whether the different pathophysiological NP-SLE mechanisms have an impact in these outcomes and in which magnitude this results would be dependent on other disease characteristics.

METHODS

Patients

Patients from the Leiden NP-SLE-clinic were used. Our study group comprised 131 SLE patients presenting at least one NP-event either related or non-related to SLE. Our hospital, the Leiden University Medical Centre, serves as a national referral centre for NP-SLE in the Netherlands. All patients fulfilled the ACR 1982 revised criteria for SLE.(12,13) For the present study, only patients with completed SF-36 questionnaires at the appropriate assessments were included. The study was approved by the local medical ethics committee. All patients have provided written informed consent.

Multidisciplinary assessment of NP-events

All patients included in our study were evaluated twice. In both visits all patients were admitted for a 1-day period. They underwent the same standardized assessment including a combination of multidisciplinary medical assessment and extensive complementary testing. During the admission all patients were assessed by a multidisciplinary team including specialists in rheumatology, neurology, clinical neuropsychology, psychiatry and vascular medicine. An experienced rheumatologist calculated SLE disease activity with the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K).(14) Irreversible damage due to SLE was assessed with the SLICC/ACR damage index (SDI) at the first visit.(15) In both cases, SLEDAI-2K and SDI were calculated without the NP variables included in these indexes. Furthermore, extensive laboratory tests, neuropsychological evaluation and a brain magnetic resonance imaging (MRI) were routinely performed. When needed additional tests, such as cerebrospinal fluid analysis or MRI of the spine were also performed. Evaluations included in the multidisciplinary assessment and MRI-scanning protocol are described elsewhere.(2,16) The multidisciplinary team met every 2 weeks to discuss the patients and evaluate the complementary assessments. The next aspects were taken into account: a) objective confirmation of symptoms assessed to standard of care of the appropriate medical specialty, b) attribution to SLE or other aetiology. Both NP-SLE and non-NP-SLE-events could coexist in the same patient, c) assessment of the suspected pathogenic mechanism when NP-SLE was diagnosed, differentiating between inflammatory and ischemic NP-SLE as previously reported.(2,16) Both phenotypes could also coexist, and d) Classification of

NP-events according to the 1999 ACR-nomenclature for NP-SLE.(17) More than one NP-SLE diagnosis per patient was possible. Thereafter, an individualized therapeutic decision per NP-event was made depending on presentation and severity.(18) In general, when inflammatory NP-SLE was suspected, immunosuppression therapy was initiated; in case of ischemic NP-SLE, secondary prevention with antiaggregants or anticoagulation when indicated was given; and when a NP-event was not related to SLE, optimization of symptomatic therapy or/and psychotherapy were indicated. Furthermore, all patients were closely followed by the referral doctor in between visits. After re-assessment, a final diagnosis was established taking into account the evolution over time of NP status and response to therapy of every NP-event.

Patient's and physician's reported outcomes of NP-events

Likert scale

A 4-point Likert scale was used to assess the clinical outcome of every NP-event between the first visit and re-assessment (1=worsening of symptoms including death; 2=no change; 3=improvement of symptoms; 4=resolution of symptoms). Likert scales have been previously used by other groups as physician reported outcome in NP-SLE studies.(11, 19) For ischemic NP-events and transverse myelitis we used the modified Rankin scale, a validated tool for evaluating disability and dependence in daily activities.(20) A positive change in the Rankin score between first visit and re-assessment of > 2 points was assessed as improvement; a negative change of ≥ 1 was assessed as worsening.

SF-36 score

The SF-36 was used as measure of HRQoL at first visit and re-assessment. All SF-36 domains and both subscales the SF-36 Physical Component Summary Score (SF-36 PCS) and the SF-36 Mental Component Summary Score (SF-36 MCS) were calculated.(21) All these scores were retrospectively calculated and not available to the multidisciplinary team and subsequently not taken into account to decide the diagnosis at re-assessment. The difference between the SF-36 MCS and PCS at the paired visits was used as dependent variable. SF-36 questionnaires were assessed per patient; however, since multiple concurrent NP-events may occur in our patients and since clinical outcome was assessed per NP-event, we decided to use also this approach to assess HRQoL. The same values of change in SF-36 MCS and PCS were used for all NP-events occurring in the same patient. Although this approach may have an impact in our results we preferred this situation over leaving out of the study patients presenting multiple NP-events of different origin.

Statistics

All data are expressed as mean (\pm standard deviation), medians with interquartile range (IQR) or proportion if applicable. All variables were normally distributed.

Firstly, independent associations between a large number of clinical-demographic variables (age, gender, disease duration, duration NP-event, lag time between SLE diagnosis and NP-event presentation, time interval between visits, change in SLEDAI-2K [cSLEDAI-2K], SDI at first visit, antiphospholipid syndrome diagnosis, NP-SLE diagnosis and NP-SLE phenotypes) were investigated by univariate linear regression analysis, using the change in Likert scale, SF-36 MCS and PCS as the dependent variables. There was not a statistical interaction between age or gender and the dependent variables. Variables with univariate associations with a $p < 0.20$ were retested in a multivariate model.

Secondly, a multiple variable analysis was performed in order to test for the contributory or confounding effect of several independent variables. The variables were included one by one in the model. Separate multivariate models were run using either NP-SLE diagnosis or NP-SLE phenotype per event as independent variables. For NP-SLE phenotype we used dummy variables for inflammatory (yes = 1, else = 0) and ischemic (yes = 1, else = 0) with non-NP-SLE-events as reference.

Furthermore, ANOVA test with Bonferroni correction was performed to compare the change in all SF-36 domains among groups. All tests were two-sided and p values < 0.05 were considered statistically significant. Statistical analysis was performed with commercially available software (IBM SPSS statistics, version 20.0 for Windows; SPSS, Chicago, IL, USA).

RESULTS

Patient's characteristics

In total 131 SLE patients had two completed SF-36 questionnaires at first visit and re-assessment. **Table 1** shows the clinical characteristics, autoantibody profile and therapies given after first visit in the study population. There were 115 women (87.8%), mostly Caucasian (70.2%) with a mean age at diagnosis of 35.61 ± 13.66 years and mean disease duration at first visit of 7.16 ± 7.72 years. SLE activity and cumulative organ damage at the first visit were moderate as showed by the mean SLEDAI-2K (8.11 ± 6.34) and SDI (1.45 ± 1.2) scores after exclusion of NP variables. The median interval between visits was 0.5 years (IQR 0.4–1.1).

Characteristics of the NP-events

A total of 232 NP-events were diagnosed at first visit. Patients presented a median number of 2 NP-events (range 1–5). A total of 120 NP-events were attributed to SLE and 112 NP-

Table 1. Characteristics at enrolment of 131 SLE patients presenting NP-events

		%
Female, n (%)	115	87.8
Age at diagnosis (years)	35,61 ± 13,66	
Age at study (years)	42,77 ± 13,02	
Ethnicity, n (%)		
Caucasian	92	70.2
Black	9	6.9
Asian	26	19.8
Mixed	3	2.3
SLE duration (years)	7,16 ± 7.72	
NP-event duration (years)	1,11 ± 2,71	
Number of SLE criteria, median (IQR)	5 (4-6)	
ACR SLE criteria, n (%)		
Malar rash	56	42.7
Discoid rash	23	17.6
Photosensitivity	42	32.1
Oral ulcers	46	35.1
Arthritis	86	65.6
Serositis	37	28.2
Renal disorder	33	25.2
Neuropsychiatric disorder	16	12.2
Hematologic disorder	64	48.9
Immunologic disorder	103	78.6
Positive antinuclear antibody	129	98.5
Antibodies, n (%)		
aCL IgG	30	22.9
aCL IgM	14	10.7
LAC	54	41.2
B2GP-1 IgG †	16	
B2GP-1 IgM †	5	
Antinuclear antibody	124	94.7
ENA	65	49.6
Anti-dsDNA	70	53.4
Anti-SSA	39	29.8
Anti-SSB	9	6.9
Anti-RNP	27	20.6
Anti-Sm	12	9.2
SLEDAI-2K *	8.11 ± 6.34	
SDI *	1.45 ± 1.2	
Therapies after first visit, n (%)		
Corticoids	76	58
Immunosuppressants	64	48.9
Cyclophosphamide	24	18.3
Rituximab	4	3.1
IVIg	1	0.8
Azathioprine	27	20.6
Mycophenolate	11	8.5
ASA	39	29.8
Dipyridamole	10	7.6
Antidepressants	28	21.4
Anticonvulsants	19	14.5
Vitamin K antagonists	30	22.9
Clopidogrel	6	4.6
Benzodiazepines	12	9.2
Antipsychotics	10	7.6
Triptans	7	5.3
Statines	22	16.8
Psychotherapy	26	19.8

aCL: anticardiolipin antibodies; ACR: American College of Rheumatology; ANA: antinuclear antibody; ASA: acetyl salicylic acid; B2GP-1: anti-β2-glycoprotein 1; IVIG: intravenous immunoglobulin; LAC: Lupus anticoagulant; NP: neuropsychiatric; NP-SLE: neuropsychiatric systemic lupus erythematosus; SLE: systemic lupus erythematosus; SDI: systemic lupus international collaborating clinics (SLICC)/American College of Rheumatology damage index; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000.

† Only 91 patients were assessed for B2GP IgG and IgM.

* Calculated without neuropsychiatric variables.

events to other aetiologies. Among the 120 NP-SLE-events a total of 74 were addressed as inflammatory NP-SLE and 46 NP-events as ischemic NP-SLE. Among the 112 non-NP-SLE-events, a total of 38 NP-events (33.9%) were concomitant in patients presenting at least one NP-SLE-event. **Supplementary Table 1** shows a description of all NP-events by attribution and according to the ACR nomenclature. Attribution to SLE varied significantly depending on the different NP-event included in this nomenclature (i.e. 85% cerebrovascular disease and 8% headaches).

Outcomes of NP-events

After re-assessment 19% of all NP-events resolved, 32.7% improved, 34.5% were unchanged and 13.8% worsened in NP status. A total of 46/120 (38.3%) NP-SLE-events improved and 35/120 (29.2%) resolved. A total of 30/112 (26.8%) non-NP-SLE-events improved and only 9/112 (8%) resolved after re-assessment. A total of 15.8% ischemic NP-SLE and 51.6% inflammatory NP-SLE improved. NP-SLE-events and especially inflammatory NP-SLE had markedly better HRQoL outcomes than ischemic NP-SLE and non-NP-SLE. **Figure 1** shows the change in the eight domains of SF-36 among 232 NP-events presenting in SLE patients depending on the final diagnosis and phenotype.

Relationship between NP-SLE diagnosis and clinical outcome, change in SF-36 MCS and SF-36 PCS

In general, NP-events attributed to SLE had better clinical outcome and a positive change in SF-36 MCS and PCS. **Table 2** shows the results of univariate and multivariate logistic (**Model 1**) regression analysis exploring the association between clinical outcome measured by Likert scale, change in SF-36 MCS and PCS, the NP-SLE diagnosis and the clinical-demographic variables of interest:

- Clinical outcome: univariate regression analysis showed that NP-SLE (regression coefficient [B] = 0.582; $p < 0.001$), age ($B = -0.011$; $p < 0.05$), Asian and mixed ethnicity (both $p < 0.05$) and cSLEDAI-2K ($B = 0.031$; $p < 0.001$) were associated with clinical outcome.
- Using multivariate analysis NP-SLE was still independently associated with clinical outcome ($B = 0.502$; $p < 0.001$) and the only important predictor was cSLEDAI-2K ($B = 0.021$; $p < 0.05$).
- SF-36 MCS: NP-SLE ($B = 8.966$; $p < 0.001$), age ($B = -0.402$; $p < 0.001$), disease duration ($B = -0.588$; $p < 0.001$) and cSLEDAI-2K ($B = 0.864$; $p < 0.001$) were associated with change in SF-36 MCS. Multivariate analysis showed that NP-SLE remained significant ($B = 5.783$; $p < 0.05$) although it was significantly influenced by adding disease duration ($B = -0.552$; $p < 0.001$) and cSLEDAI-2K ($B = 0.705$; $p < 0.001$) to the model.

- SF-36 PCS: NP-SLE diagnosis ($B=6.086$; $p<0.05$), age ($B=-0.297$; $p<0.05$) and cSLEDAI-2K ($B=0.493$; $p<0.05$) were associated with change in SF-36 PCS. However, after multivariate analysis only age showed still a negative association ($B=-0.216$; $p<0.05$).

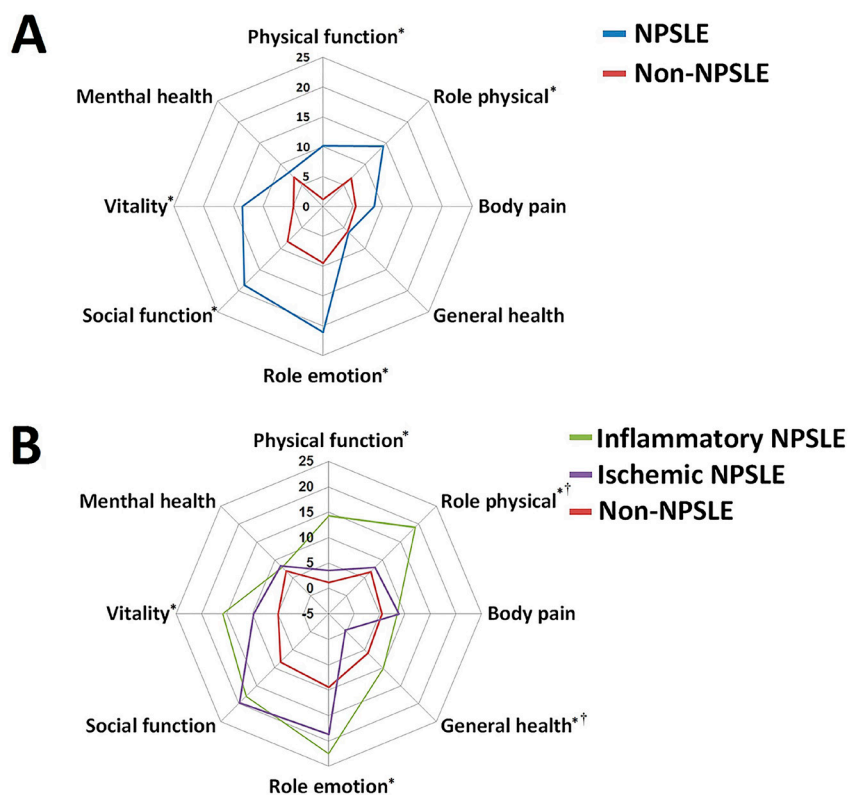


Figure 1. Spidergram representing the change in HRQOL of the 8 domains of SF-36 among 232 NP-events presenting in SLE patients depending on its pathogenesis. **A.** Comparison between NP-events attributed to SLE (NP-SLE) and to other aetiologies (Non-NP-SLE). * $p < 0.05$; **B.** comparison between NP-SLE events attributed to inflammation (inflammatory NP-SLE), to ischemia (ischemic NP-SLE) or to other aetiologies (Non-NP-SLE). * $p < 0.05$ for comparison between inflammatory-NP-SLE and non-NP-SLE; † $p < 0.05$ for comparison between inflammatory NP-SLE and ischemic NP-SLE.

Table 2. Univariable and multivariable logistic regression analysis exploring the association between clinical outcome, change in SF-36 MCS, SF-36 PCS (dependent variables), attribution of NP-events according to diagnosis and phenotype and clinical-demographic variables

Clinical Outcome (Likert)		Change in SF-36 MCS		Change in SF-36 PCS	
Univariable	Multivariable	Univariable	Multivariable	Univariable	Multivariable
	Model 1 – NP-SLE diagnosis Estimate (95% CI)	Model 2 – NP-SLE phenotype Estimate (95% CI)	Model 1 – NP-SLE diagnosis Estimate (95% CI)	Model 1 – NP-SLE diagnosis Estimate (95% CI)	Model 2 – NP-SLE phenotype Estimate (95% CI)
Diagnosis NP-SLE	0.582 (0.374 to 0.817)**	0.502 (0.257 to 0.747)**	8.966 (4.115 to 13.817)**	5.783 (1.017 to 10.548)*	6.086 (0.997 to 11.174)*
Phenotype NP-SLE					
Non-NP-SLE (Ref.)					
Inflammatory NP-SLE	0.894 (0.640 to 1.149)**	0.827 (0.560 to 1.093)**	14.715 (9.395 to 20.035)**	11.133 (5.866 to 16.400)**	6.133 (0.319 to 11.948)*
Ischemic NP-SLE	0.080 (-0.218 to 0.378)	0.028 (-0.272 to 0.328)	-0.283 (-6.502 to 5.936)	-2.075 (-8.016 to 3.866)	3.337 (-2.712 to 9.385)
Age at enrolment (years)	-0.011 (-0.020 to -0.001)*	-0.003 (-0.013 to 0.007)	-0.402 (-0.594 to -0.210)**	-0.176 (-0.373 to 0.021)	6.009 (-0.788 to 12.806)
SLE duration (years)	-0.001 (-0.018 to 0.015)		-0.588 (-0.913 to -0.262)**	-0.138 (-0.329 to 0.054)	-0.216 (-0.428 to -0.004)*
NP-event duration (years)	-0.022 (-0.078 to 0.033)		-0.552 (-0.865 to -0.238)**	-0.237 (-0.581 to 0.106)	-0.219 (-0.433 to -0.006)*
			-1.098 (-2.218 to 0.022)	-0.237 (-0.581 to 0.106)	0.168 (-0.997 to 1.333)

Gender (Female vs Male)	-0.271 (-0.636 to 0.095)	-0.268 (-7.698 to 7.162)	-3.608 (-11.262 to 4.047)
Ethnicity			
Caucasians (Ref.)			
Black	0.360 (-0.436 to 1.156)	-4.908 (-21.162 to 11.347)	7.289 (-9.528 to 24.105)
Asian	0.780 (0.077 to 1.482)*	-2.645 (-16.991 to 11.700)	7.530 (-7.311 to 22.371)
Mixed	0.147 (0.013 to 0.282)*	-1.395 (-4.145 to 1.356)	1.792 (-1.054 to 4.637)
cSLEDAI-2K †	0.031 (0.012 to 0.049)**	0.021 (0.002 to 0.039)*	0.020 (0.002 to 0.038)*
		0.021 (0.002 to 0.039)*	0.705 (0.342 to 1.069)**
		0.864 (0.502 to 1.226)**	0.690 (0.339 to 1.041)**
			0.493 (0.106 to 0.879)*
SDI at enrolment †	-0.032 (-0.134 to 0.069)	-1.853 (-3.886 to 0.180)	1.739 (-0.362 to 3.840)
APS (Yes vs No)	-0.127 (-0.409 to 0.156)	-2.495 (-8.210 to 3.221)	-2.743 (-8.641 to 3.155)
Time between visits (years)	-0.062 (-0.163 to 0.038)	-0.588 (-2.604 to 1.488)	-1.106 (-3.214 to 1.002)

APS: antiphospholipid syndrome; MCS: mental component summary score; PCS: physical component summary score; NP: neuropsychiatric; NP-SLE: neuropsychiatric systemic lupus erythematosus; cSLEDAI-2K: change of SLEDAI-2K (Systemic Lupus Erythematosus Disease Activity Index 2000) between first visit and re-assessment; SF-36: 36-item Short-Form Health Survey. *P < 0.05, **P < 0.001; † Calculated without neuropsychiatric variables

Relationship between NP-SLE phenotypes and clinical outcome, change in SF-36

MCS and SF-36 PCS

We further investigated the association between the outcome variables and the underlying pathophysiological mechanism of NP-events and whether the association was independent of differences in clinical-demographic variables (**Table 2, Model 2**):

- Clinical outcome: only inflammatory NP-SLE was significantly associated with a favourable outcome ($B=0.894$, $p<0.001$), remaining significant in a multivariate analysis ($B=0.827$; $p<0.001$). Multivariate analysis showed that cSLEDAI-2K was a significant predictor ($B=0.020$, $p<0.05$) of this association.
- SF-36 MCS: an association between inflammatory NP-SLE ($B=14.715$; $p<0.001$) and improvement in SF-36 MCS was observed, remaining significant after multivariate analysis ($B=11.133$; $p<0.001$). Disease duration ($B=-0.525$, $p<0.001$) and cSLEDAI-2K ($B=0.690$; $p<0.001$) accounted as strong additional potential predictors.
- SF-36 PCS: inflammatory NP-SLE was significantly associated with an improvement in SF-36 PCS ($B=6.133$, $p<0.05$); after multivariate analysis only age was associated with change in SF-36 ($B=-0.216$, $p<0.05$).

DISCUSSION

Our results show that inflammatory NP-SLE-events have a better clinical outcome and a meaningful improvement in SF-36 MCS than non-NP-SLE-events and ischemic NP-SLE-events. Moreover we show that SLE disease activity is key as predictor of these results.

We propose that these findings may be related to reversibility of brain inflammation/dysfunction after starting immunosuppressive therapy as well as to spontaneous decrease of disease activity. Inflammatory NP-SLE reflects neuronal dysfunction or brain inflammation thought to be mediated by autoantibodies, other inflammatory factors and increased SLE disease activity. Histopathological studies in NP-SLE have shown findings compatible with inflammation (e.g. parenchymal oedema, glial hyperplasia).(22) Furthermore, studies using quantitative MRI have reported a parallel improvement of clinical status and cerebral changes in white matter of NP-SLE patients after receiving immunosuppressive therapy.(16) Reversibility of symptoms after immunosuppressive therapy has been also described in other immune mediated diseases of the central nervous system presenting with a heterogeneous group of NP symptoms such as anti-NMDA-receptor encephalitis.(23)

The results of our study show that only inflammatory NP-SLE may explain the better clinical outcome in NP-SLE found by other authors.(6,7) Ischemic NP-SLE-events, mainly represented by patients with cerebrovascular symptoms, improve slightly over time after starting secondary prevention, which may indicate the irreversibility of cumulative chronic damage on the brain.(16)

Previous research has shown that the focal events have better clinical outcome and higher resolution when compared with diffuse NP-events.(7) Our results do not support these data; probably due to the different inclusion of NP-SLE-events in these subgroups (focal and diffuse vs ischemic and inflammatory), suggesting that both approaches are not comparable. For example, the SLICC-cohort includes seizure in the focal group while in most seizures included in our study an inflammatory mechanism was suspected. We suggest that a differentiation per NP-event based in the underlying pathophysiological mechanism may be preferable since it can be used to guide therapeutic decisions. The presence of NP-events in SLE patients, independently of the aetiology, is associated with a significant HRQoL burden.(5-7) Our study confirms these results and shows how almost all SF-36 variables have a positive change at re-assessment in all groups independently of the origin of NP-events. Previous research has found that the mean SF-36 MCS is markedly lower in SLE patients presenting with NP-events, especially in diffuse NP-events.(6) We show similar results, principally for inflammatory NP-SLE; the subsequently meaningful positive change in the mean SF-36 MCS in this group may respond to the fact that these patients have more room for improvement.

We may speculate that certain NP-events included in this group, such as acute confusional state, may have an important impact in our results, since they lead to a more impaired clinical status. The change in HRQoL is slightly higher than in previously reported(7), suggesting that multidisciplinary assessment and therapeutically orientated interventions per NP-event may be a good approach in SLE. Further studies are warranted to evaluate and identify which specific therapeutic interventions may be required to improve the outcome of different subgroups in NP-SLE. In general, our results support the hypothesis of reversibility in inflammatory NP-SLE and therefore the use of SF-36 as outcome in future clinical trials.

Our data suggest that the change in disease activity measured without neuropsychiatric variables plays an important role determining both clinical outcome and change in SF-36 MCS. Previous studies in SLE without NP-events have shown greater reductions in disease activity accompanied by a meaningful improvement in HRQoL measures after starting immunosuppression.(24,25) In SLE patients presenting with NP-events, an association between lower mean SF-36 MCS and higher disease activity has been observed.(7) In the current study, disease activity does not interfere with the association between NP-SLE-events and both clinical outcome and change in SF-36 MCS, especially inflammatory NP-SLE-events, which may reflect a direct effect of immunosuppression. Moreover, our results show that SF-36 MCS is also influenced by disease duration. Patients who are in the early stages of the disease and are diagnosed with NP-SLE will have more positive change in SF-36 MCS than later in the disease, which may imply that longer disease leads to a burden in brains of SLE patients.

Our study has limitations. First of all, since response to medication influences the attribution of NP-events to SLE, especially in inflammatory NP-SLE, we may not avoid a certain circular reasoning in these studies until we have more specific tools to diagnose NP-SLE. Other limitation and also a generally recognized problem in NP-SLE studies is the high heterogeneity of the NP syndromes which leads to a low prevalence of individual NP-SLE syndromes, which does not allow us to know in which magnitude our results conducted by a certain NP-SLE syndrome. Our results represent a single-centre experience. However, an advantage comparing with other studies is that patients underwent the same multidisciplinary assessment, so far the best and most trustable method to reach NP-SLE diagnosis. Other limitation is that due to the impaired clinical status of some NP-SLE patients we had to postpone the fulfilling of the SF-36. Moreover, due to referral matters, some of the inflammatory NP-SLE patients were evaluated soon after they had been started with immunosuppression. Therefore, we believe that probably the change may have been even higher than those reported here. Furthermore, it is unknown what would have happen if all patients would have strictly been seen every 6 months or after longer periods of follow-up. To avoid bias at this point, time between visits was used as an independent variable in the multivariate analysis.

In conclusion, our results show for the first time that inflammatory NP-SLE-events have a better clinical and patient's reported outcome than non-NP-SLE and ischemic NP-SLE-events, reflecting reversibility of brain inflammation and improvement of disease activity after starting immunosuppression. We believe that these outcomes are helpful as measurements of SLE burden on the brain and follow-up of these patients; subsequently they can be used for monitoring of future therapy NP-SLE trials.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Description of all NP-events presenting in 131 SLE patients by attribution and according to the ACR nomenclature *

ACR definition	Diagnose		Total
	Non-NP-SLE	NP-SLE	
Cerebrovascular disease	7	40	47
Psychosis	5	7	12
Headache	23	2	25
Mood disorder	36	7	43
Myelopathy	1	10	11
Cognitive dysfunction	25	21	46
Seizure	7	12	19
Anxiety	4	2	6
Acute confusional state	0	7	7
Movement disorder	1	4	5
Aseptic meningitis	0	1	1
Polyneuropathy	2	3	5
Cranial neuropathy	0	2	2
Mononeuropathy	1	0	1
Autonomic disorder	0	1	1
Plexopathy	0	1	1
	112	120	232

ACR: American College of Rheumatology

*Possible >1 NP-SLE event per patient

10

SUMMARY AND CONCLUSIONS

Adapted from *Front Med.* 2018 Dec 4;5:340.
Adapted from *Drugs.* 2016; 76: 459-83.

SUMMARY, CONCLUSIONS AND FUTURE

PERSPECTIVES

The studies included in this thesis focus in different aspects of the pathogenesis, diagnosis and outcome of neuropsychiatric (NP) manifestations presenting in patients with systemic lupus erythematosus (SLE). In particular the following topics were addressed. First, laboratory biomarkers, specifically serum complement cascade and autoantibodies, and their associations with NP-SLE manifestations and pathophysiological changes as seen on magnetic resonance imaging (MRI) were analyzed. Second, the role of quantitative neuroimaging techniques in the detection of brain microstructural changes and in the identification of underlying pathophysiological process in NP-SLE was assessed. Third, the value of multidisciplinary re-assessment of patients in the attribution of NP manifestations to SLE or other etiologies was evaluated and its role as gold standard in the diagnostic process of NP-SLE was discussed. Finally, we investigated if the clinical and patient's related outcomes of NP manifestations presenting in SLE were associated with a different underlying pathophysiological process.

This final chapter summarizes the main findings of the studies comprised in this thesis and suggests potential future research paths to address important unmet needs in the NP-SLE field. The factors that need to be addressed to improve the diagnostic or attribution process of NP manifestations presenting in SLE patients and the extent to which new therapies may impact the future treatment of NP-SLE will also be discussed.

Summary and conclusions

Laboratory biomarkers

To start, in **Chapter 2**, we presented the story of a case of C1q deficiency associated with severe inflammatory and ischemic NP-SLE. C1q deficiency, a rare immunodeficiency, is probably the strongest susceptibility factor for the development of SLE and so far the only deficiency of early components of the complement classical pathway (C1q/C1r/C1s, C2, or C4) where NP involvement is present (20%).⁽¹⁾ A mutation in the C1qC-gene can either lead to complete deficiency or to low C1q levels with C1q polypeptide in the form of low-molecular weight (LMW) C1q. We showed how the serum of our patient contained very low levels of a non-functional LMW variant of C1q due to a homozygous G34R mutation in the C1qC-gene. We also provided a literature overview of NP-SLE in C1q deficiency and showed how these patients present with more severe forms of NP-SLE, mainly presenting with seizures and neuroimaging changes in basal ganglia and cerebral vasculitis, than in complement competent NP-SLE patients. We hypothesized about the potential role of C1q in the genesis

of nervous involvement in SLE and the subsequent presentation of NP-SLE manifestations. Therefore, we concluded that the classical pathway is not necessary to develop NP-SLE; however the absence of C1q and, subsequently, some of its biological functions may be associated with NP-SLE and a more severe presentation.

Decreased levels of complement components, complement activation and higher levels of antibodies against C1q (anti-C1q) are a hallmark of active SLE.(2) In **Chapter 3** we studied the relationships between serum levels of anti-C1q, C1q circulating immune complexes (CIC), complement activation and complement components in SLE patients during the first NP-SLE manifestation. We showed an association between focal NP-SLE and a decreased C4 and between diffuse NP-SLE and markedly decreased activation of the alternative pathway (AP50), a decreased C3 and higher levels of anti-C1q antibodies. Posterior multivariate analysis showed that these associations may be explained due to other factors such as antiphospholipid antibodies (aPL) in the case of focal NP-SLE and global disease activity in the case of diffuse NP-SLE. Importantly, among the individual NP-SLE syndromes, AP50 and C3 were markedly decreased in lupus psychosis and cognitive dysfunction, which warrants further research.

The association between serum antibodies and NP-SLE manifestations was analyzed in **Chapter 4**. As a novelty we examined autoantibody clusters and we used an addressable laser bead immunoassay test for the detection of multiple SLE specific autoantibodies. Four separate clusters of autoantibody profiles were identified: Cluster 1 no specific autoantibodies, Cluster 2 anti-dsDNA/anti-SSA/anti-SSB/anti-TRIM21, Cluster 3 anti-Sm/RNP and Cluster 4 anti-dsDNA/lupus anticoagulant (LAC)/anticardiolipin (aCL) IgM/IgG.

In our present study we found an association between Cluster 4 and NP-SLE, which was consistent with available literature.(3, 4) This association was especially important in major focal NP-SLE manifestations (cerebrovascular disease, chorea, seizures and myelopathy) and was stronger when patients with minor NP-SLE syndromes (headache, anxiety, cognitive dysfunction, and mild forms of depression) were excluded. An association between other autoantibodies analyzed with the microarray kit or clusters of these autoantibodies and NP-SLE manifestations were not found.

Neuroimaging biomarkers

There is an imperative need of finding radiological techniques that help highlighting a certain underlying pathogenic processes (ischemic or inflammatory) and subsequently guide therapy in NP-SLE. In **Chapters 5-7** we investigated whether the pathophysiological changes as seen on neuroimaging are associated to underlying immune abnormalities in SLE and whether these techniques are helpful to identify different subsets of NP-SLE.

The association between serum autoantibodies with specific brain-MRI abnormalities was analyzed in **Chapter 5**. Furthermore, we studied whether these structural changes were associated with other SLE-related or classical cardiovascular disease (CVD) risk factors. Serum autoantibodies tested were LAC, aCL IgG and IgM, anti-dsDNA, anti-SSA/Ro-52, anti-SSB/La, anti-Sm, anti-RNP and thereafter assessed individually and in groups (total number of autoantibodies). We demonstrated the lack of association between the total number and individual SLE-related autoantibodies with inflammatory-like lesions while the total number of antiphospholipid antibodies, especially the positivity for LAC, were associated with several ischemic brain abnormalities and cerebral atrophy. Furthermore, cumulative SLE-organ damage and modifiable CVD risk factors, such as hypertension, contribute to these ischemic changes pointing out the importance of systemic accelerated atherosclerosis in SLE. In order to reassure our results, the effect of the clinical neuropsychiatric status and a sensitivity analysis including Beta-2-Glycoprotein 1 antibodies IgG and IgM in the analysis were performed.

In **Chapter 6**, we assessed magnetic transfer imaging (MTI) in a prospectively followed cohort of SLE patients presenting NP symptoms either related or unrelated to SLE, to investigate whether these parameters may highlight different pathogenic NP-SLE processes (inflammatory or ischemic). Among the MTI parameters, previous research in small groups of NP-SLE patients demonstrated that magnetization transfer ratio (MTR) histogram peak height (HPH) can be used as a quantitative estimate of tissue microstructural integrity in the brain. (5, 6) We have applied MTR-HPHs in the white matter and grey matter of different NP-SLE subgroups including healthy controls, SLE, non-SLE related NP symptoms and NP-SLE. The last group was also divided into inflammatory NP-SLE and ischemic NP-SLE according to the suspected pathogenic mechanism. We demonstrated for the first time that white matter MTR-HPHs might provide evidence for the presence of inflammatory NP-SLE. We found that inflammatory NP-SLE patients have lower white matter MTR-HPH values when compared with ischemic NP-SLE, SLE patients without ever NP symptoms, non-SLE related NP symptoms and healthy controls. Moreover, in a prospective level and as previously suggested,(7) we confirmed how white matter MTR-HPH is sensitive to clinical changes, highlighting its potential role as radiologic biomarker in the diagnostic process and follow-up of NP-SLE patients and with the monitoring of future treatment trials.

Cell-specific microstructural alterations in the brain of SLE patients with and without history of NP-SLE were investigated in **Chapter 7**. To this aim we used a 7-T MRI scanner to acquire T1-weighted images, diffusion tensor imaging (DTI) datasets, and single volume diffusion-weighted magnetic resonance spectroscopy (DW-MRS) data from the anterior body of the corpus callosum. We showed how intracellular alterations and particularly changes in glia, as shown by an increase in the average diffusivities of total choline and total creatine,

significantly correlated with past NP-SLE and SLE activity. We suggested that diffusion properties of choline compounds and of total creatine are potentially unique markers for glial reactivity in response to inflammation and remarked the great potential of DW-MRS for the study of the aetiology of disease related changes in tissue microstructure of patients with SLE/NP-SLE.

Improving attribution of neuropsychiatric manifestations in SLE

The correct attribution of NP events to systemic lupus erythematosus or to an alternative etiology remains a challenge. Besides being a crucial issue, with important implications for management and prognosis of these patients, studies analyzing rigorously the attribution of NP events to SLE are very scarce. Several attribution models for NP events occurring in SLE have been proposed; however multidisciplinary expert physician judgment based on clinical and complementary tests remains the most reliable reference standard for NP-SLE diagnosis.^(8, 9) The contribution of reassessment in the attribution process of NP events to SLE or other etiologies was addressed in **Chapter 8**. We showed how in clinical practice NP events presenting in SLE are too often attributed to an immune mediated origin and how re-assessment increases diagnostic accuracy in NP-SLE. Moreover, according to our data clinical judgment cannot be substituted by any of the current attribution models available so far. We showed how, until we find more reliable tests, clinical follow-up and re-assessment of these patients will remain as reference standard in NP-SLE diagnosis.

The clinical outcome of NP events presenting in SLE has been poorly studied. In **Chapter 9**, we analyzed in detail the clinical outcome and change in health-related quality of life (HRQoL), measured by the 36-item Short Form Health Survey (SF-36), of NP events either related and non-related to SLE and whether the different pathophysiological NP-SLE mechanisms (inflammatory or ischemic) had an impact on these outcomes. We showed that inflammatory NP-SLE events have a better clinical outcome and a meaningful improvement in SF-36 mental component summary score than non-NP-SLE and ischemic NP-SLE events. Importantly, SLE disease activity was key as predictor of these results. Our results reflect the reversibility of brain inflammation and the improvement of disease activity after starting immunosuppression. Therefore, we proposed that these outcomes are helpful as measurements in the follow-up of NP-SLE patients and for monitoring future therapy NP-SLE trials.

Challenges and future perspectives in the diagnosis of NP-SLE

Yet despite years of efforts of the NP-SLE scientific community, the number of clinically useful biomarkers and even of validated biomarkers is embarrassingly modest. A series of scientific challenges in the field have yet to be overcome:

Laboratory biomarkers

- *Identification of new neuronal surface antigens* responsible for NP-SLE. The antigen identification paradigm which has been successfully used with limbic encephalitis may be applied on NP-SLE to recognize unknown neuronal cell-surface protein(s).(10) Determination of neuronal immunoreactivity in different areas of brain and cerebellum of homogeneous clinical and radiological NP-SLE groups may be analyzed and afterwards correlated with clinical symptoms and MRI characteristics. To identify the target antigen cultured neurons and mass spectrometry should be used. Lastly, brains of knock out animal models or cells deprived of the suspect antigen by siRNA knock down may confirm the specificity of these candidate autoantibodies.(11)

- *Complement cascade and IFN- α* : the exciting area of research in NP-SLE mice models on complement cascade and IFN- α need to be translated to human NP-SLE. The study of these two biomarkers may lead to a better understanding of pathogenic underlying mechanisms of synapse loss and will probably open the door to the use of new therapeutic strategies in NP-SLE.

- *Blood brain barrier (BBB)*: The BBB is a network of endothelial cells and pericyte and astrocyte projections that regulates the entry of soluble molecules and cells into the brain parenchyma. It has been proposed that a disruption of the integrity of the BBB may have a potential pathogenic role in NP-SLE since this may permit the influx of neuropathic antibodies across the BBB. Brain tissue-reactive antibodies in NP-SLE are thought to be synthesized in the CNS, but also in peripheral organs (lymph nodes and bone marrow). In the last case it was proposed that these autoantibodies must pass through the BBB of SLE patients to exert an effect upon neurons. Although an important role of BBB has been supposed, we need better understand the BBB in human NP-SLE and the factors disrupting this barrier. Studies comparing serum and CSF and using quotients are warranted.

Neuroimaging biomarkers

- *Another look at conventional MRI (cMRI) – the case for more sophisticated characterization of lesions*: In recent years, characterization of lesions in other neurological disorders mimicking NP-SLE has advanced far beyond the basic lesion count or lesion load. A notable example is the work related to lesions and their pathological classification in multiple sclerosis (MS), a well-known mimicker of NP-SLE. The latest and most significant step in characterization of white matter lesions in MS came after examining the spatial relationship between lesions and large veins in a visualization method that superimposes FLAIR images containing lesion spatial information, and T₂*-weighted images, showing vein distribution in great detail.(12) It was found that concentric co-localization of a white matter lesion with a vein that passes through it, termed central vein sign (CVS), is highly specific to the early stages of MS and

has been swiftly adopted as a biomarker mandated for MS diagnosis by the North American Imaging in Multiple Sclerosis Cooperative (NAIMS). The presence and development of CVS are well explained by a neuroinflammatory mechanism with a vascular origin, and CVS has been shown to differentiate well between MS and other central nervous system inflammatory vasculopathies including SLE(13) but not patients with active NP-SLE.

- *quantitative MRI (qMRI) – magnetization transfer imaging as biomarker in NP-SLE?* Histograms of qMRI values provide a versatile and sensitive tool which is commonly applied in neurological disorders.(14, 15) They are sensitive to diffuse, global effects, and successive studies have shown the sensitivity of magnetization transfer ratio (MTR) histograms, an MTI-derived parameter, to a variety of disease related clinical and laboratory factors.(5) As several studies have highlighted, MTR is a potential marker for brain microstructural changes in NP-SLE. However, there are still several questions that must be elucidated in the near future. First, MTR histograms provide a cumulative estimation of a quantitative measure, and thus lack any spatial information. Most studies, including ours presented in Chapter 6, focused on analysis of whole brain or tissue-specific (gray matter, white matter) histograms of MTR values. Studies on SLE using this technique will need to focus on specific brain regions, either selected as a region of interest or a specific brain structure (e.g. basal ganglia). Second, MTR values are known to be low in both gray and white matter in patients with NP-SLE. The mechanism by which these values are decreased is not clear, but its reversibility upon successful treatment suggests intracellular edema and gliosis as the associated pathophysiological changes.(16) However further studies are required to fully determine whether these data reflect these changes on the brain or whether they represent the severity of NP symptoms apart from the SLE.

- *Same picture – multiple views: the role of multimodal neuroimaging in NP-SLE:* The multifactorial nature of NP-SLE, combined with the lack of specificity of most imaging modalities to any particular pathomechanism makes the quest for a “silver bullet” diagnostic tool unrealistic. A natural approach is to combine several neuroimaging markers, each highlighting a different aspect of the disease in an approach that uses a multivariate analysis in one way or the other. Several approaches for multimodal data analysis of neuroimaging data have been proposed for other neuropsychiatric and neurological disorders, especially for those with little overt brain damage and complex underlying mechanisms such as major psychiatric disorders. The application of these analyses in NP-SLE patients may help to phenotype patients and elucidate several of the underlying mechanisms.(17-19)

Improving attribution of neuropsychiatric manifestations in SLE

- *NP-SLE definition.* Since 1999, research in this field has been guided by the ACR case definitions for NP-SLE syndromes including a group of nineteen complex and uncommon

neuropsychiatric manifestations involving both the central (12 syndromes) and peripheral (7 syndromes) nervous system.(20) Researchers have mainly focused on analyzing biomarkers in NP-SLE defined as a group based in these definitions without taking into account the underlying pathophysiological mechanism. Using such heterogeneous manifestations as a group may be problematic since it may include manifestations with obviously different underlying pathophysiological mechanisms, i.e. stroke and acute confusional state. Clinicians and researchers in the field would benefit from resolving the problem of heterogeneity and the use of biomarkers capturing the different aspects of nervous involvement in SLE. Borowoy et al. demonstrated how autoantibody associations depend on the NP-SLE definition used.(21) In clinical practice, the gold standard is a diagnosis conducted by a multidisciplinary expert clinical team. Furthermore, the diagnosis in NP-SLE is made phenotypically according to the suspected underlying pathophysiological mechanism since is extremely important for guiding treatment.(22) Phenotypic characterization is important in clinical practice but may be also in research. A given phenotype may arise from a diverse set of biochemical processes and its changes in the brain may be captured by a diverse set of neuroimaging techniques. The identification of a biochemical subset of factors that underlie a certain phenotype or a certain NP-SLE manifestation should be preferable in future research.

- *Small sample size due to the low prevalence* is one of the common denominators of studies describing new potential biomarkers in NP-SLE. Given the complexity of NP-SLE, collaborative efforts, using pooled clinically, laboratory and neuroimaging data sets are needed. Much larger studies will allow for more specific hypothesis about for example a specific phenotype or NP-SLE manifestation or for example permit the use of biomarker combinations and analyze the relations among them.

- *Study design NP-SLE vs. SLE.* A reason for the minimal clinical impact of reported biomarkers may be that most of these studies report differences between NP-SLE patients and SLE at a group level while physicians have to make clinical decisions individually. Furthermore, in clinical practice, when a SLE patient presents with NP complaints obligates first to exclude other potential causes before these symptoms are attributed to SLE or to other etiologies. Most of the studies compare the higher presence of a certain biomarker in SLE patients with and without NP-SLE manifestations, remaining uncertain if this biomarker profiles are unique to NP-SLE or may be present in other mimicking neuropsychiatric disorders; only a few studies have used a group of patients with other neuropsychiatric disease (i.e. MS or septic meningitis) as control groups.(23) For example, B-cell activating factor of TNF family or matrix metalloprotease-9 have been proposed as exploratory biomarker in NP-SLE because its higher positivity when compared with SLE; however both biomarkers have been also validated as biomarkers in patients with MS.(24)

- *Omics*: in the last years, laboratory biomarker discovery has benefit from the development of omics technologies such as genomics or immune-proteomics, which has successfully increased the list of exploratory biomarkers in many diseases.(25) These techniques give the opportunity to explore a wide spectrum of biomarkers in a more comprehensive and unbiased way. Autoantigen microarrays have already been used in NP-SLE.(26, 27) For example, van der Meulen et al. have shown how a profile of IgG and IgM autoantibodies against 15 antigens may help to differentiate NP-SLE from non-NP-SLE.(26) The potential for false positive discoveries using these techniques is high; reproduction of this data and selection of best candidates may be a next step before validation in large-scale independent cohorts.(28)

- *Machine learning*: the application of the previous techniques in NP-SLE will produce hundreds of exploratory biomarkers. Analytical methods such as supervised *machine learning* (ML) promise help solving this problem and advance the development of biomarkers in the near future. This technique uses algorithms to automatically extract information from data that can be applied at the individual level to make predictions therefore with a higher level of clinical translation. This technique can be applied to laboratory biomarkers but also to neuroimaging data, since ML methods are sensitive to spatially distributed and subtle effects.(29)

Challenges and future perspectives in the treatment of NP-SLE

Drugs in Development

Advances in the understanding of immunopathogenesis of SLE have led to the development of immunotherapies targeting B cells, T cells, the costimulatory modulation, and cytokines. Although pathogenic mechanisms in NP-SLE are still poorly understood and experimental models using these new therapies are lacking, we could speculate about the potential role of some of these drugs in the future treatment of these manifestations. The promising effect of rituximab, a chimeric monoclonal antibody directed against the B-cell-specific antigen CD20, may suggest an important contribution of B cells to NP-SLE pathogenesis. Belimumab, a humanized monoclonal antibody targeted against B lymphocyte stimulator (BLyS), is now licensed in the US and Europe for the management of SLE. The BLISS trials were neither designed nor powered to definitively demonstrate the efficacy of belimumab in specific organ systems. Other trials on therapies targeting BLyS, such as tabalumab (phase II) and blisibimod (phase III) are ongoing. Atacicept, a humanized fusion protein that binds BLyS and APRIL (a proliferation-inducing ligand) has also been tested in SLE patients.(30) Both BLyS and APRIL were shown to be elevated in the CSF of SLE patients. Furthermore, they are produced locally in the astrocytes. Hence, antagonists of these cytokines could have beneficial effect in these patients; however, patients with severe CNS manifestations were excluded from all these trials, which will limit any conclusion in this respect.(31)

Several drugs targeting cytokines that are thought to contribute to the pathogenesis of both SLE and NP-SLE are currently being tested. For example, IFN- α is considered one of the most promising therapeutic targets in SLE. Sifalimumab, a human anti-IFN- α monoclonal antibody, and rontalizumab, a humanized monoclonal antibody IgG1, have shown promising results in reducing SLE disease activity across multiple clinical measures.(32) Although not confirmed in all studies, IFN- α is one of the inflammatory mediators related to NP-SLE pathogenesis. Type I IFNs are found in glia and neurons. Among their functions, IFNs induce other inflammatory mediators such as IL-6, alter brain neurotransmitters such as serotonin, and generate brain toxic metabolites. Subsequently, IFN- α has been hypothesized as a potential target in NP-SLE.(33) However, in most of trials, CNS involvement was an exclusion criterion, and the potential to treat NP-SLE will remain unknown.(31) Several studies have confirmed the intrathecal presence of higher levels of other cytokines (tumor necrosis factor (TNF)- α , IL-6, and IFN- γ) in NP-SLE. The overproduction of these cytokines is thought to play a role in the pathogenesis and severity of NP symptoms, and they have been proposed as candidate targets for future treatment.(34) Ischemic NP-SLE, especially in the presence of aPL or antiphospholipid syndrome (APS), may benefit from new-generation direct oral anticoagulants in the future, including dabigatran etexilate, a direct thrombin inhibitor, and rivaroxaban, apixaban and edoxaban, which are direct anti-Xa inhibitors.(35) Although not currently recommended in APS, these therapies may represent a potential alternative for long-term anticoagulation in APS. Rivaroxaban has shown good results in both arterial and venous thrombosis; however, information is controversial [191, 192].(36, 37) More data will be drawn from ongoing studies.

Potential Future Targets

Many modulators of the integrity of the BBB have been proposed as a potential future target to treat NP-SLE. Among them, anti-endothelial cell antibodies, complement components, cytokines and chemokines, and environmental mediators have an essential role.(38) It has been speculated that ameliorating the disruption of the BBB may have an important effect in the control of NP-SLE. Studies in MRL/lpr mice, accurately reflecting human NP-SLE, have shown the importance of TWEAK, a pro-inflammatory cytokine member of the TNF superfamily, and the alternative complement cascade in BBB disruption. TWEAK variably induces cellular proliferation, angiogenesis, apoptosis, and the production of metalloproteinase, cytokines, and chemokines.(39) TWEAK has been found to be increased in the cerebral cortices of MRL/lpr mice. Furthermore, in a murine knockout model for its receptor Fn14, mice were found to improve in cognitive function and to have less depression and anhedonia.(40) Complement component C5 has been reported to play a role in the maintenance of the BBB in mice.(41) Selective inhibition of C5aR alleviated CNS lupus.(42) Also, inhibition of the classical and alternative complement cascade with the complement inhibitor Crry was

demonstrated to alleviate experimental CNS lupus in mice.(43) Furthermore, complement plays a role in microvascular injury. Mice deficient in C3 and C5 components are resistant to enhanced thrombosis and endothelial cell activation induced by aPL antibodies, indicating the important role of alternative pathway complement activation on aPL antibody-mediated thrombogenesis.(44) Based on this information, eculizumab, a humanized monoclonal antibody blocking the generation of terminal complement components C5a and C5b-9, may be a potential drug to be used in the future in NP-SLE.(45)

Final comments

In this thesis we have analyzed an important number of laboratory, radiological, clinical and patient's reported outcomes in SLE patients presenting with NP manifestations. Our studies are among the most robust to date in this field due to the large number of patients included, the prospective character and the standard assessment followed by a multidisciplinary expert consensus. Furthermore our studies include the novelty of a phenotypic characterization of all NP manifestations according to the suspected underlying pathophysiological mechanism (inflammation or immune-mediated vs. ischemic or thrombotic). Our studies have given more light to the understanding of the underlying pathophysiological mechanisms of nervous involvement in systemic lupus erythematosus.

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11

SAMENTVATTING EN CONCLUSIES

NEDERLANDSE SAMENVATTING EN CONCLUSIES

Eén van de belangrijke functies van het afweersysteem (immuunsysteem) is het lichaam te wapenen tegen binnendringende ziektekiemen, zoals virussen en bacteriën. Dit doet het lichaam onder andere door het aanmaken van antilichamen en het aansturen van witte bloedcellen (de immuunrespons). Antilichamen zijn eiwitten die 'vreemde' cellen herkennen en zich aan deze cellen binden om ze vervolgens te kunnen laten opruimen. Onder normale omstandigheden is de immuunrespons alleen gericht tegen lichaamsvreemde cellen. Het komt echter voor dat het immuunsysteem cellen van het eigen lichaam aanvalt en zo voor onnodige weefselschade zorgt. Deze inadequate immuunrespons tegen lichaamseigen cellen heet auto-immuniteit. De antilichamen die de lichaamseigen cellen 'aanvallen' heten auto-antilichamen. Er bestaan, voor zo ver tot op heden bekend is, meer dan 100 verschillende auto-immuunziekten.

Systemische lupus erythematoses (SLE) is zo'n (chronische) auto-immuunziekte. Het komt voor bij personen van iedere etnische afkomst, alle leeftijden en zowel mannen als vrouwen, maar meer dan 90% van de SLE patiënten die gediagnosticeerd worden zijn vrouwen in de vruchtbare leeftijd (15 tot 44 jaar). Patiënten kunnen uiteenlopende klachten hebben doordat bijna ieder orgaan van het lichaam door SLE aangetast kan worden. SLE kenmerkt zich door aanwezigheid van veel verschillende auto-antilichamen.

Net als andere organen, zoals nieren, hart en longen, kan ook het zenuwstelsel aangetast worden door SLE. Ziektebetrokkenheid van het zenuwstelsel kan leiden tot een diverse groep van neurologische en psychiatrische uitingen (zie **Tabel 1** in **Hoofdstuk 1**), ook bekend als neuropsychiatrische systemische lupus erythematoses (NP-SLE). Verondersteld wordt dat deze uitingen vaak bij SLE voorkomen en voor een slechte prognose zorgen met een 10 keer zo hoog risico op overlijden ten opzichte van de algemene bevolking.

Er is slechts weinig bekend over de onderliggende mechanismen die tot NP-SLE leiden, oftewel hoe het immuunsysteem gezond hersen- of zenuwweefsel aantast, en de daaruit voortkomende veranderingen (**pathofysiologie**) in de hersenen en het ruggenmerg. Aangezien de ziekte gekenmerkt wordt door het aanmaken van auto-antilichamen, is het aannemelijk om te denken dat deze antilichamen bijdragen aan het ontstaan van NP-SLE. Er wordt gedacht dat het centraal zenuwstelsel doelwit is van één of meerdere auto-antilichamen en dat de aanwezigheid van die bepaalde auto-antilichamen tot NP-SLE kunnen leiden. Naast de auto-antilichamen worden er bij laboratorium onderzoek nog allerlei andere afwijkingen, biomarkers, gevonden die vermoedelijk ook een bijdrage leveren in het ontstaan van NP-SLE. Biomarkers zijn meetbare stoffen in het lichaam die iets kunnen zeggen over gezondheid of ziekte. De biomarkers die genoemd zijn in het

kader van NP-SLE betreffen meerdere spelers (eiwitten en moleculen) in het veld van het immuunsysteem. Er is echter nog onvoldoende kennis over de associatie tussen het ontstaan van NP-SLE en de aanwezigheid van de auto-antilichamen en/of andere biomarkers.

Naar aanleiding van de vermoedelijke onderliggende ontstaanswijze kan NP-SLE in twee groepen worden verdeeld. De twee verschillende pathofysiologische processen die tot neuropsychiatrische symptomen leiden bij SLE patiënten zijn: (1) inflammatoir, geassocieerd met ontstekingsstimulerende en/of auto-immuun gemedieerde oorzaken; en (2) ischemisch/trombotisch, gerelateerd aan vernauwing/obstructie van bloedvaten (bijvoorbeeld herseninfarct). Deze inflammatie of ischemie van het centraal zenuwstelsel leidt tot verminderd functioneren, zich uitend in neurologische en/of psychiatrische klachten. Neuropsychiatrische klachten kunnen optreden door beschadiging/ontsteking van een specifiek gebied in de hersenen (focaal), zoals bij een infarct of bij epilepsie, maar kunnen ook door afwijkingen die over bijna de gehele hersenen verspreid zijn (diffuus), zoals hoofdpijn en cognitieve beperkingen. Op beeldvorming van de hersenen van NP-SLE patiënten, bijvoorbeeld op MRI scans, wordt schade gezien waardoor neuropsychiatrische klachten verklaard zouden kunnen worden. De afwijkingen op hersenscans hebben het begrip over de pathofysiologische veranderingen in NP-SLE vergroot en helpen bij het maken van beslissingen aangaande de diagnose en behandeling (zie **Figuur 1** in **Hoofdstuk 1**).

Ondanks de inspanningen van veel onderzoekers is tot op heden geen van de biomarkers uit het laboratorium of de neurologische beeldvorming specifiek voor de diagnose NP-SLE. Dat betekent dat er geen tests bestaan om de ziekte te kunnen aantonen. Er blijft daardoor een grote behoefte bestaan om biomarkers te ontwikkelen die op betrouwbare wijze verschillende aspecten van de ziekte representeren. In de klinische praktijk is het moeilijk om neuropsychiatrische klachten bij SLE patiënten te duiden en op de juiste manier te behandelen. Dit komt doordat neuropsychiatrische symptomen zeer divers zijn en niet specifiek zijn voor de ziekte SLE. Met andere woorden: de neuropsychiatrische klachten kunnen door SLE veroorzaakt worden maar kunnen ook een andere oorzaak hebben. Zo kan bijvoorbeeld een psychose door activiteit van SLE veroorzaakt worden óf kan het uitgelokt worden door bepaalde medicijnen óf als uiting van een ander primair probleem optreden. Het is van belang om hiertussen onderscheid te maken omdat het bepalend is voor de behandeling. NP-SLE is een **diagnosis per exclusionem**, dit houdt in dat alle andere bekende oorzaken van de specifieke neuropsychiatrische klacht uitgesloten moeten worden voordat de diagnose NP-SLE gesteld kan worden. Tot nu toe is de beste manier om NP-SLE aan te tonen, dan wel uit te sluiten, een multidisciplinair teamoverleg nadat de patiënt uitgebreid gezien is door verschillende specialisten en meerdere onderzoeken heeft ondergaan.

De behandeling van NP-SLE is veelal gebaseerd op klinische ervaring in plaats van op wetenschappelijk bewijs doordat er relatief weinig studies van hoge kwaliteit zijn gedaan. De behandeling die wordt ingezet is ofwel gericht tegen inflammatie ofwel tegen het voorkomen van ischemie (herseneninfarcten), of een combinatie van beide. Bij het vermoeden op inflammatoire NP-SLE, of wanneer de SLE actief is in andere organen wordt gestart met medicatie die het immuunsysteem onderdrukken (immunosuppressiva zoals corticosteroïden). Het doel van de therapie is om de klachten te verhelpen of in ieder geval te stabiliseren. Bij de verdenking op een herseneninfarct in het kader van ischemische SLE wordt gestart met antistollingstherapie om de kans op een herhaling in de toekomst te verkleinen.

Leiden NP-SLE cohort

De studies die in dit proefschrift opgenomen zijn bevatten patiënten uit het Leiden NP-SLE cohort. Het Leids Universitair Medisch Centrum is een landelijk NP-SLE centrum waar patiënten vanuit andere ziekenhuizen naar verwezen worden als hun dokter de diagnose NP-SLE overweegt. Alle patiënten in dit cohort hebben dus SLE én neuropsychiatrische klachten. Iedere patiënt doorloopt een gestandaardiseerd programma, het NP-SLE Zorgpad. De zorg georganiseerd als zorgpad houdt in dit geval in dat alle medische consulten, het neuropsychologisch onderzoek, de 3-tesla MRI-scan van de hersenen, het laboratoriumonderzoek en eventueel ander aanvullend onderzoek op dezelfde dag plaatsvinden (zie **Tabel 2** in **Hoofdstuk 1**). Het team van het NP-SLE Zorgpad is multidisciplinair en bestaat uit specialisten van verschillende afdelingen: Reumatologie, Neurologie, Psychiatrie, Neuropsychologie, Vasculaire geneeskunde en Neuroradiologie. Deze specialisten komen tweewekelijks bij elkaar om de rol van SLE bij de betreffende neuropsychiatrische klachten te duiden op basis van alle verkregen onderzoeksresultaten. Op basis van de kennis en expertise van al deze specialismen tezamen kan een goed onderbouwde (meest waarschijnlijke) diagnose gesteld worden en een daarbij passend behandelplan. Na 3 tot 18 maanden vindt een herevaluatie plaats. Patiënten komen dan opnieuw één dag in het LUMC om hetzelfde zorgpad te doorlopen.

Het proefschrift

In dit proefschrift zijn onderzoeken opgenomen die verschillende aspecten van NP-SLE beschrijven; van moleculair onderzoek tot patiëntenzorg. Deze onderzoeken worden verdeeld in 3 onderdelen:

Allereerst; uit laboratoriumonderzoek verkregen biomarkers, in het bijzonder de complement cascade en auto-antilichamen, en hun associaties met NP-SLE uitingen en pathofysiologische veranderingen zoals gezien worden op **magnetic resonance imaging (MRI)**.

Ten tweede; de rol van beeldvormingstechnieken van het centraal zenuwstelsel in het aantonen van kleine structurele veranderingen in de hersenen en de daaraan ten grondslag liggende pathofysiologische processen bij NP-SLE.

Als laatste; de bijdrage van de herevaluatie van NP-SLE patiënten in het verklaren van NP klachten en de rol van het multidisciplinair team als huidige gouden standaard bij de diagnostiek van NP-SLE. Tot slot werd er onderzocht of onderliggende pathofysiologische processen van NP-SLE tot een verschil in uitkomsten leidt.

SAMENVATTING EN CONCLUSIES

Laboratorium biomarkers

Het complementsysteem speelt een belangrijke rol bij het verdedigingsmechanisme van het immuunsysteem. Het complementsysteem bestaat uit een reeks eiwitten die elkaar als een soort kettingreactie activeren en zo een ontstekingsreactie teweeg brengen. Het helpt onder andere bij het afbreken van ziektekiemen die in eerste instantie zijn herkend door antilichamen. Het complementsysteem wordt onderverdeeld in drie verschillende activatiepaden; de klassieke-, de lectine- en de alternatieve route (Zie **Figuur 2** in **Hoofdstuk 1**). Recente onderzoeken bij muizen toonden aan dat het complementsysteem, vooral de klassieke route, een cruciale rol speelt bij het ontstaan van verstoorde functies van het centraal zenuwstelsel en de klachten die patiënten als gevolg daarvan ervaren.

C1q, het eiwit waarmee de kettingreactie van de klassieke route begint, lijkt een hoofdrol te spelen in het proces naar neuropsychiatrische klachten. Als C1q ontbreekt ontstaat er een verstoring binnen het immuunsysteem waardoor individuen een verhoogd risico op infecties hebben en daarnaast ook een hoger risico op het ontwikkelen van auto-immuunziekten.

C1q deficiëntie is een zeldzame aandoening waarbij er sprake is van een C1q tekort. Dit ziektebeeld is tot nu toe de sterkste voorspeller voor het ontwikkelen van SLE. Patiënten met C1q deficiëntie hebben in 20% van de gevallen neuropsychiatrische betrokkenheid van de ziekte. Een mutatie in het C1qC-gen kan leiden tot een lage concentratie van, een non-functioneel, C1q in het bloed of tot volledige afwezigheid van het eiwit. In **Hoofdstuk 2** wordt een patiënt-casus beschreven waarin een link wordt gelegd tussen een C1q deficiëntie en ernstige NP-SLE (inflammatoir en ischemisch). In het bloed van deze patiënt werd een lage concentratie non-functioneel C1q gevonden veroorzaakt door een bepaalde mutatie in het C1qC-gen. In dit hoofdstuk staat ook een overzicht over NP-SLE bij patiënten met C1q deficiëntie weergegeven, gebaseerd op de beschikbare literatuur. Het lijkt er op dat patiënten met C1q deficiëntie een meer ernstige vorm van NP-SLE hebben dan patiënten

met NP-SLE zonder C1q deficiëntie. Vooral epilepsie, ontsteking van de bloedvaten in de hersenen en afwijkingen op hersenscans in een specifieke regio, de basale kernen, worden vaker gezien bij deze patiëntengroep. We veronderstelden een rol voor C1q in het optreden van centraal zenuwstelsel betrokkenheid bij SLE patiënten. Concluderend is disfunctie van de klassieke complement route geen noodzakelijke stap in het ontwikkelen van NP-SLE, maar C1q-deficiëntie is wel geassocieerd met NP-SLE en een meer ernstige klinische uitingsvorm hiervan.

Verlaagde concentraties van eiwitten uit het complementsysteem, activatie van de complement routes en verhoogde concentraties van antilichamen tegen C1q (anti-C1q) zijn kenmerken van actieve SLE. In **Hoofdstuk 3** wordt dieper ingegaan op de relatie tussen anti-C1q, immuuncomplexen, complement activatie en andere complement componenten bij SLE patiënten die hun eerste NP-SLE episode doormaken. We vonden een aantal associaties tussen het hebben van NP-SLE en een verlaagde concentratie van verschillende complement eiwitten, verminderde activatie van de alternatieve route en verhoogde spiegels van C1q-antilichamen. Een nadere analyse die rekening hield met meerdere factoren liet zien dat andere factoren deze associaties mogelijk (gedeeltelijk) verklaarden. Deze factoren betroffen de aanwezigheid van zogeheten antifosfolipiden antilichamen en een hoge algemene ziekteactiviteit van SLE. Wat verder opviel zijn de lage concentraties van complement factor C3 en activeringsroute AP50 die gevonden werden in NP-SLE patiënten met psychoses of cognitieve achteruitgang. Er is meer onderzoek nodig om deze relatie verder op te helderen en te bevestigen.

Ook al is de aanmaak van verschillende auto-antilichamen kenmerkend voor SLE, is er nog geen directe causale relatie gelegd tussen deze antilichamen en specifieke NP-SLE klachten of tussen de antilichamen en afwijkingen op MRI scans van de hersenen. De associaties tussen de aanwezigheid van antilichamen in het bloed en NP-SLE uitingsvormen werd geanalyseerd in **Hoofdstuk 4**. We gebruikten een clusteranalyse om te kijken of verschillende auto-antilichamen vaak gezamenlijk voorkomen en er zo verschillende clusters gezien konden worden. We konden 4 clusters onderscheiden bij SLE patiënten; Cluster 1 bestond uit patiënten zonder auto-antilichamen, Cluster 2 uit de antilichamen anti-dsDNA/anti-SSA/anti-SSB/anti-TRIM21, Cluster 3 uit anti-Sm/RNP en Cluster 4 uit anti-dsDNA/lupus anticoagulant (LAC)/anticardiolipin (aCL) IgM & IgG. De eerste drie clusters toonden geen verschil tussen SLE en NP-SLE maar het vierde cluster toonde wel een associatie met NP-SLE. Deze associatie tussen de antilichamen uit cluster 4 en NP-SLE komt overeen met wat in eerdere literatuur beschreven is. Deze relatie is het grootst in focale NP-SLE uitingen zoals infarcten en epilepsie en werd nog sterker na het excluderen van minder ernstige NP-SLE uitingen. Tussen de individuele auto-antilichamen, buiten de individuele antilichamen uit cluster 4, werden geen associaties met NP-SLE uitingen gevonden.

Beeldvorming van de hersenen

In **Hoofdstukken 5-7** onderzochten we in hoeverre de op hersenscans geziene veranderingen waren geassocieerd met onderliggende immunologische veranderingen bij SLE. Daarnaast werd onderzocht in hoeverre beeldvorming van de hersenen bijdraagt in het onderscheiden van verschillende NP-SLE subtypes.

In **Hoofdstuk 5** werd de associatie tussen auto-antilichamen en afwijkingen op MRI scans van de hersenen geanalyseerd. Verder onderzochten we in dit hoofdstuk ook of deze MRI afwijkingen geassocieerd zijn met andere risicofactoren. Het gaat hierbij om zowel SLE-gerelateerde risicofactoren als de bekende cardiovasculaire risicofactoren. De auto-antilichamen die in deze studie beoordeeld zijn, waren LAC, aCL IgG, aCL IgM, anti-dsDNA, anti-SSA/Ro-52, anti-SSB/La, anti-Sm en anti-RNP. Deze werden in relatie tot de MRI afwijkingen zowel per antilichaam individueel vergeleken als in groepen antilichamen (som van totaal aantal antilichamen). Er werd geen associatie gevonden tussen inflammatoire MRI-afwijkingen en de verschillende auto-antilichamen. Tussen de aan ischemie gerelateerde MRI-afwijkingen en specifieke auto-antilichamen werd wel een associatie gevonden. Deze specifieke auto-antilichamen heten als groep antifosfolipiden, ze bestaan uit o.a. LAC, aCL IgG en aCL IgM. Ook het totaal aantal antifosfolipide antilichamen die bij één patiënt aanwezig zijn toont een associatie met ischemische afwijkingen op de MRI, waarbij een groter aantal een grotere associatie laat zien. Daarnaast dragen cumulatieve schade in (verschillende) organen door de SLE en het hebben van cardiovasculaire risicofactoren ook bij aan de ischemische veranderingen op de MRI's van de hersenen. Dit suggereert dat versnelde aderverkalking waarschijnlijk een grote rol speelt in schade die ontstaat in SLE.

In **Hoofdstuk 6** onderzochten we een eventuele bijdrage van kwantitatieve beeldvormingstechnieken bij NP-SLE patiënten. Het doel was te achterhalen of deze technieken een verschil in onderliggende pathofysiologie kon aantonen: wel/geen NP-SLE en inflammatoir/ischemisch. In deze studie werden naast de NP-SLE patiënten en SLE patiënten met (ongerelateerde) neuropsychiatrische klachten ook gezonde personen en SLE patiënten zonder neuropsychiatrische klachten als controle geïnccludeerd. De techniek waarover het hier gaat is magnetic transfer imaging (MTI) waarbij gebruik gemaakt wordt van de kwantitatieve waarden: magnetic transfer ratio (MTR) en histogram peak height (HPH). De resultaten van het onderzoek toonden verlaagde MTR-HPH waarden in de witte stof van inflammatoire NP-SLE patiënten ten opzichte van de ischemische NP-SLE, SLE met en zonder NP klachten en de gezonde controlegroep. Het was een prospectief onderzoek waarbij de MTR-HPH opnieuw werd berekend tijdens herevaluatie. Als uitkomst werd gevonden dat MTR-HPH verandert als ook de klachten veranderen. Deze resultaten suggereren een mogelijke rol van MTR-HPH als radiologische biomarker in het diagnostisch proces van NP-SLE. Het kan in de

toekomst dan wellicht ook gebruikt worden om het resultaat van behandelingen te monitoren.

Microstructurele, cel-specifieke veranderingen in de hersenen van SLE patiënten mét en zónder voorgeschiedenis van NP-SLE werden onderzocht in **Hoofdstuk 7**. Voor dit onderzoek gebruikten we een scanner met een extra hoge resolute, een 7-tesla MRI scanner, om zo T1-gewogen beelden, **diffusion tensor imaging** (DTI) en **single volume diffusion-weighted magnetic resonance spectroscopy** (DW-MRS) data te verkrijgen van een specifiek hersengebied, de voorzijde van de corpus callosum (de hersenbalk). We zagen hoe veranderingen op celniveau, voornamelijk veranderingen in de gliacellen (speciale hersencellen die ondersteunende functies hebben voor het hersenweefsel), significant correleerden met in het verleden doorgemaakte NP-SLE en SLE activiteit. De veranderingen die middels DW-MRS gezien werden waren een toename in de gemiddelde diffusie van de stoffen choline en creatine. We veronderstellen dat de veranderingen in diffusie eigenschappen van choline en creatine potentieel unieke biomarkers zijn voor het aantonen van reactivatie van gliacellen in reactie op inflammatie. Hiermee draagt de DW-MRS ook bij aan het bestuderen van de ontstaanswijze van structurele schade in de hersenen van patiënten met SLE/NP-SLE.

Verbeterd onderscheid tussen neuropsychiatrische symptomen wel of niet veroorzaakt door SLE

De precieze attributie van neuropsychiatrische uitingen aan SLE is niet geheel opgehelderd. Ondanks de noodzaak van het correct diagnosticeren van NP-SLE, wat vooral van belang is in relatie tot de behandeling en prognose, is er weinig onderzoek gedaan in dit veld. Verschillende modellen om tot de diagnose NP-SLE te komen (attributiemodellen) zijn gepubliceerd, maar tot nu toe blijft de expert opinion van een multidisciplinair team de gouden standaard. In **Hoofdstuk 8** behandelen we de eventuele waarde van de herevaluatie van patiënten die 3-18 maanden eerder het zorgpad hebben doorlopen. Het effect van de ingezette behandeling na 3-18 maanden kan in retrospect een ander licht schijnen op de diagnose. De herevaluatie helpt bij het vergroten van de diagnostische nauwkeurigheid bij NP-SLE. Uit de studie beschreven in dit hoofdstuk blijkt dat neuropsychiatrische klachten te vaak onterecht toegeschreven werden aan inflammatoire NP-SLE. In retrospect gekeken werd minder vaak de diagnose inflammatoire NP-SLE gesteld dan bij eerste bezoek aan het zorgpad. De beschikbare attributiemodellen kunnen ook in het Leiden NP-SLE cohort de expert opinion niet vervangen. Totdat er meer betrouwbare tests beschikbaar zijn blijven we afhankelijk van het multidisciplinaire klinische zorgpad en de herevaluatie bij het diagnostisch proces.

Er is weinig bekend over het verloop en de prognose van de neuropsychiatrische symptomen bij SLE patiënten. In **Hoofdstuk 9** analyseerden we de verandering in gezondheid-gerelateerde kwaliteit van leven (HRQoL) bij het tweede bezoek aan het NP-SLE Zorgpad ten

opzichte van het eerste bezoek. Dit werd gemeten met een gestandaardiseerde vragenlijst, de 36-item Short Form Health Survey (SF-36) die beide keren werd ingevuld. Dit onderzoek toonde aan dat patiënten met neuropsychiatrische klachten als gevolg van inflammatoire NP-SLE een grotere verbetering ondervonden in de kwaliteit van leven na behandeling dan de patiënten met neuropsychiatrische klachten ten gevolge van ischemische NP-SLE of ten gevolge van een andere oorzaak dan SLE. De belangrijkste voorspeller van de kwaliteit van leven was de ernst van de SLE ziekte activiteit op het moment van invullen. Deze resultaten geven indirect aan dat het starten van immunosuppressieve therapie zinvol is en ontsteking/inflammatie in de hersenen omkeerbaar is. We concluderen dat het meten van bepaalde uitkomsten, zoals kwaliteit van leven en ziekteactiviteit, nuttig zijn om te evalueren na starten van behandeling in het kader van NP-SLE. Daarnaast is de herevaluatie een goed moment voor het monitoren van het effect van een ingestelde behandelingen.

Tot slot

In dit proefschrift werden een groot aantal meetbare uitkomstwaarden onderzocht bij SLE patiënten met neuropsychiatrische klachten. Deze uitkomstenwaarden bevatten zowel gegevens uit laboratoriumonderzoek als radiologische scans en klinische gegevens zoals resultaten van neuropsychiatrische tests en door de patiënt gerapporteerde resultaten. De studies die gedaan zijn binnen het Leiden NP-SLE cohort behoren tot de meest gerenommeerde onderzoeken in het veld van NP-SLE door het relatief grote aantal patiënten, het vervolgen van de patiënten in de tijd en het gestandaardiseerde zorgpad. Verder omvatten deze onderzoeken de uitsplitsing van de twee NP-SLE subtypen: ischemisch/trombotisch en inflammatoir/ontstekings-gemedieerd, iets wat uniek is binnen het onderzoeksveld. De in dit proefschrift gerapporteerde onderzoeken hebben voor meer inzicht gezorgd in de onderliggende mechanismen die leiden tot het optreden van neuropsychiatrische symptomen bij SLE patiënten.

Appendices

LIST OF PUBLICATIONS

Publications included in this thesis

1. Magro-Checa C, Kumar S, Ramiro S, Beart-van der Voorde LJ, Eikenboom J, Ronen I, de Bresser J, van Buchem MA, Huizinga TW, Steup-Beekman GM. Are serum autoantibodies associated with brain changes in systemic lupus erythematosus? - MRI data from the Leiden NP-SLE cohort. *Lupus*. 01/2019
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9. Magro-Checa C, Zirkzee E, Huizinga TW, Steup-Beekman GM. Management of Neuropsychiatric systemic lupus erythematosus: current approaches and future perspectives. *Drugs*. 03/2016.
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CURRICULUM VITAE

César Magro Checa was born on the 30th of April 1983 in Guadalajara, Spain. He studied at the Arcipreste de Hita School in Azuqueca de Henares, Spain, where he graduated in 2001. He studied Medicine at the University of Alcalá in Spain. During his studies he did a rotation of 4 months in León, Nicaragua and 1 year of practical internships at the University Hospital of the Ruhr-University of Bochum, Germany. He obtained his medical degree in June 2007. He trained in Rheumatology at the Department of Rheumatology of Hospital Universitario San Cecilio, in Granada, Spain, under the supervision of Dr. Enrique Raya Alvarez and Dr. Juan Salvatierra Ossorio. As part of his training he did a 3-month rotation at the Department of Rheumatology of Hospital 12 de Octubre, in Madrid, Spain, and a 3-month rotation in clinical research at the Department of Rheumatology of Leiden University Medical Center, in Leiden, The Netherlands. He obtained his specialist accreditation in Rheumatology in May 2013. Since October 2013 until November 2017 he combined clinical work and research at the Leiden University Medical Center. Since January 2017 until August 2018 he worked as a rheumatologist in the HagaZiekenhuis, The Hague, The Netherlands. Since September 2018 he works in the Zuyderland Ziekenhuis, Heerlen and Sittard-Geleen in the Netherlands.

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