Systemic lupus erythematosus : from diagnosis to prognosis

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Chapter 6

Brain Histopathology in Patients with Systemic Lupus Erythematosus: Identification of Lesions associated with clinical Neuropsychiatric Lupus Syndromes and the Role of Complement

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Rheumatology, 2017; 56: 77-86.

ABSTRACT

Background

Neuropsychiatric (NP) involvement is a poorly understood manifestation of systemic lupus erythematosus (SLE). We studied post-mortem histopathology in relation to clinical NP-SLE syndromes and complement deposition in brains of NP-SLE and SLE patients, and control cases. Furthermore, we investigated the correlation between cerebral post-mortem histopathology and ex vivo 7-Tesla MRI findings in SLE and NP-SLE patients.

Methods

A nationwide search for autopsy material yielded brain tissue from 16 NP-SLE and 18 SLE patients. Brains obtained from 24 patients who died from acute cardiac events served as controls. Apart from a histopathologic evaluation, paraffin-embedded tissue from the cerebral cortex was stained for components of the classical and lectin complement pathways, as well as for the terminal complement complex.

Results

Diffuse vasculopathy, microinfarction, macroinfarction, vasculitis, and microthrombi occurred significantly more often in NP-SLE than SLE, and were absent in controls. Focal vasculopathy was found both in SLE patients and controls. Complement deposition was strongly associated with both SLE and NP-SLE, but not with controls ($P<0.001$). Microthrombi were found uniquely in NP-SLE, and were associated with C4d and C5b-9 deposits ($P<0.05$). 7-Tesla MRI was unable to detect most small vessel injury that was visible histopathologically.

Conclusions

Our study demonstrates that histopathologic lesions in NP-SLE represent a continuum, ranging from nonspecific lesions such as focal vasculopathy, to more specific lesions such as C4d- and C5b-9-associated microthrombi and diffuse vasculopathy related to clinical syndromes defining NP-SLE. Complement deposition may be a key factor in the interaction between circulating autoantibodies and thromboischemic lesions observed in NP-SLE. In conclusion, complement inhibition may have novel therapeutic potential in NP-SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a severe autoimmune disease characterised by circulating autoantibodies and immune complexes. SLE can manifest in virtually any organ system.[1] Nervous system involvement in SLE is commonly referred to as neuropsychiatric SLE (NP-SLE),[2] and occurs in 10–80% of SLE patients.[3-7] NP-SLE can present with a variety of symptoms, including stroke and psychosis, which are frequently under-recognised despite being associated with increased morbidity and mortality.[8, 9] Because NP-SLE is clinically heterogeneous, and because we lack aetiologic insight and evidence-based therapeutic interventions, the clinical management is complex.

As part of the clinical workup of a potential NP-SLE patient, magnetic resonance imaging (MRI) of the brain plays a prominent role,[10] although interpretation of scans is often hampered by the so-called "clinicoradiological paradox", by which some patients with SLE and severe neurologic symptoms exhibit no or nonspecific abnormalities on MRI. In an analysis of MRI findings from 74 NP-SLE patients, 42% had no MRI abnormalities, despite having signs and symptoms of active cerebral disease. Otherwise, white matter hyperintensities were the most common MRI finding, which has been suggested to represent cerebral hypoperfusion and infarction.[11]

The few studies performed to date regarding histopathology of NP-SLE revealed that most lesions are related to ischemic injury in the vicinity of small-diameter vessels.[12-16] Specifically, microthrombosis, microinfarction, and microbleeds were generally found. Whereas SLE is characterised by autoantibody-mediated inflammation, the findings in the brain suggest a thromboischemic pathogenesis. Thus far, studies in patients have failed to provide clues for the interaction between the autoantibody-mediated inflammation and thromboischemic lesions in NP-SLE. Given the recent evidence that classical complement activation corresponds with the presence of glomerular microthrombi in lupus nephritis[17] and other thrombotic microangiopathies,[18-22] we hypothesised that complement activation may also underlie thromboischemic injury typically observed in brains of NP-SLE patients.

In a nationwide study using cerebral autopsy material from NP-SLE, SLE, and control cases, we examined post-mortem histopathology in relation to clinical NP-SLE syndromes and complement deposition. Furthermore, in order to evaluate whether histopathologic lesions could be detected clinically, we investigated the correlation between cerebral post-mortem histopathology and ex vivo 7-Tesla MRI in SLE and NP-SLE patients.

METHODS

Nationwide search for cerebral tissues from SLE patients

We conducted a nationwide search for cerebral autopsy tissue obtained from SLE patients with and without clinical signs of neuropsychiatric involvement. Samples were searched using the Dutch PALGA database, a histo- and cytopathology network and archive founded in 1971 to which all pathology laboratories within the Netherlands contribute.[23] Hence, our search included patients autopsied between 1971–2010. We excluded samples from patients who had cutaneous or discoid lupus only, and included all patients with SLE according to the 1982 American College of Rheumatology (ACR) revised criteria[24,25] with cerebral autopsy tissue available.

Clinical data were collected by contacting the rheumatologists at the different locations where the patients had been hospitalised. Because banking of sera and cerebrospinal fluid was not routinely performed as part of the clinical workup for the patients in this study, analysis of these materials was not feasible. Patients were divided into the following groups by two rheumatologists with extensive experience in diagnosing NP-SLE (GMSB and TWJH): patients with neuropsychiatric syndromes attributable to SLE (NP-SLE group) and SLE patients without neuropsychiatric syndromes attributed to SLE (SLE group). The presence of neuropsychiatric syndromes in each patient was evaluated according to the ACR definitions,[2] and the existence of secondary factors causing these manifestations was assessed.

Controls were identified from the archives of the Leiden University Medical Center and the Reinier de Graaf Hospital (Delft, the Netherlands) and included previously healthy patients who died of an acute cardiac event (confirmed at autopsy) between 2006–2009.

Histopathology and immunohistochemistry

Sections were stained with haematoxylin and eosin using standard protocols. The following cerebral complement components were stained: C1q (classical pathway), Mannose Binding Lectin (MBL, lectin pathway), C4d, and C5b-9 (membrane attack complex). Deparaffinised sections were subjected to antigen retrieval using EDTA-TRIS (pH 9.0), 10 mM citrate buffer (pH 6.0), or proteinase (bacterial, type XXIV; Sigma-Aldrich, St. Louis, Missouri). The sections were then stained with antibodies against C1q (Dako Cytomation, Denmark, 1:800), C4d (Biomedica Gruppe, Austria, 1:50), and MBL (Sigma-Aldrich Biotechnology, 1:500), and C5b-9 (A239; Quidel, San Diego, California; 1:500). Staining was visualised using the appropriate secondary antibodies with diaminobenzidine as chromagen. Finally, sections were counterstained with haematoxylin. Optimal antibody dilutions and incubation times were predetermined empirically by titration experiments using positive controls.

Histopathologic and immunohistochemical evaluation

All sections were evaluated by a neuropathologist (SGvD) who was blinded with respect to clinical data. Each case was scored for the presence of microinfarction, macroinfarction, large haemorrhage, microbleeds, cerebral infection, vasculitis, and vasculopathy. Infarction was defined as sharply delimited regions of cellular death or tissue necrosis, sometimes with cavitation; the distinction between microinfarction and macroinfarction was made based upon the ability to detect these lesions by light microscopy or by gross inspection, respectively.[26] Infarction could be observed in the absence of (micro)thrombi. Microthrombi were defined as microscopic clumps of fibrin, platelets, and erythrocytes.[27] Microbleeds were defined as small (<5 mm) perivascular haemosiderin-deposits (usually within macrophages), generally associated with local vessel wall damage. Large haemorrhage was defined as rupture of the blood vessel wall and extravasation of erythrocytes without inflammation in acute stages and oedema, ischemia, infiltration of neutrophils, haemosiderin-laden macrophages, and necrosis in later stages.[27] Vasculitis was defined as an inflammatory infiltrate and destructive change in the blood vessel wall.[27] In contrast, vasculopathy was defined as endothelial cell proliferation, thickening of the vessel wall, and narrowing of the capillary lumen without an inflammatory infiltrate.[15] Vasculopathy was scored semi-quantitatively as "absent" (<10% of vessels with vasculopathy per low-power field [LPF]), "focal" (10–50% of vessels with vasculopathy per LPF), or "diffuse" (>50% of vessels with vasculopathy in every LPF).

The immunohistochemically stained sections were scored by two independent observers who were blinded with respect to the clinical data. C1q and C4d primarily stained endothelial cells of small vessels in the white and grey matter, and were scored as "absent" (<10% of small vessels with C1q or C4d per LPF), "focal" (10–50% of small vessels with C1q or C4d per LPF), or "diffuse" (>50% of small vessels with C1q or C4d in every LPF). C5b-9 similarly stained vessels, as well as cells with the morphology of glial cells; thus, scoring for vessels was performed similar to C1q and C4d. Because MBL did not stain vessels, MBL-positive cells were scored as being either present or absent.

Whole formalin-fixed brains: Clinical case histories

From three patients, whole formalin-fixed brains were available, enabling a direct comparison between post-mortem ex vivo MRI and cerebral histopathology. Patient 1 was a 57-year-old female with NP-SLE, antiphospholipid syndrome, and severe cerebrovascular disease. Patient 2 was a 38-year-old male with NP-SLE, acute neurologic deterioration, and vasculitis. Patient 3 was a 63-year-old-female with SLE without neuropsychiatric symptoms during the course of her disease who suffered from acute myocardial infarction. Detailed case histories are provided in **Appendix 6.1**.

Post-mortem neuroimaging and evaluation of the acquired images

The procedure of post-mortem 7-Tesla MRI scanning is detailed in **Appendix 6.2**. MRI scans were reviewed by two neuroradiologists with extensive experience in the field of

NP-SLE (MAvB and BJE), who then identified areas of interest.

Histopathologic analysis of post-mortem MRI-scanned brains

After radiological analysis, tissue blocks of areas of interest were sampled, embedded in paraffin, and stained with haematoxylin and eosin. The neuropathologist reported the histopathologic changes in each sample.

Ethical considerations

The ethics committee of the Leiden University Medical Center approved this study (P02.028). In accordance with the Dutch National Ethics guideline (Code for Proper Secondary Use of Human Tissue, Dutch Federation of Medical Scientific Societies), all tissue samples and patient data were coded and kept anonymous throughout the study.

Statistical analyses

Categorical variables were compared using chi-square test and its trend version for ordered categories (linear-by-linear analysis). Correlations between histopathologic variables were assessed by calculation of Pearson or Spearman correlation coefficients, as appropriate. All analyses were performed using SPSS Statistics 23.0 (IBM, Armonk, New York). *P*-values <0.05 were considered significant.

RESULTS

Our nationwide search for cerebral autopsy tissue of SLE patients yielded 296 hits. Of these, 48 fulfilled our inclusion criteria. From 14 of these patients, paraffin blocks were no longer available, or tissue quality was unsuitable for analysis. Thus, formalin-fixed tissue samples from 34 SLE patients (27 females, 7 males) autopsied between 1981−2009 were retrieved from 12 Dutch pathology laboratories and used for analysis. Twenty-four patients who died of an acute cardiac event were included as controls. **Table 1** provides an overview of clinical characteristics of NP-SLE, SLE, and control cases.

Three patients in the SLE group had neuropsychiatric syndromes that could be attributed to factors other than SLE. Specifically, one patient suffered intracerebral haemorrhage associated with anticoagulant therapy, one patient had a severe cerebral mycotic infection associated with high-dose immunosuppressive therapy for lupus nephritis, and one patient died in a uraemic coma following acute renal failure secondary to lupus nephritis.

Histopathology

The majority of histopathologic lesions were located in the cerebral cortex, distributed equally across the white and grey matter. The patient groups (NP-SLE and SLE) differed significantly from the controls with respect to all histopathologic parameters. Microinfarction ($P=0.016$), macroinfarction ($P=0.002$), and vasculitis ($P<0.050$) were more frequent

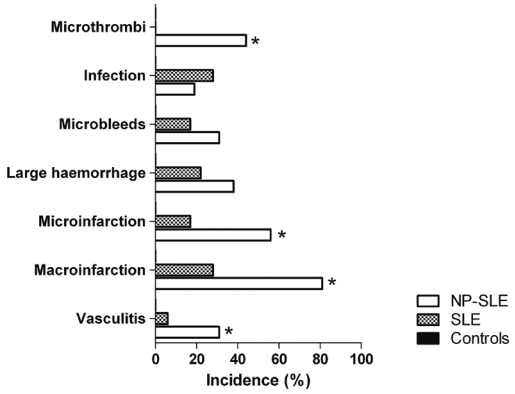
in NP-SLE patients than SLE patients. Microthrombi were found exclusively in NP-SLE patients (P=0.002, versus the other groups). Histopathologic findings in the three groups are shown in **Figure 1**.

Table 1 Patient characteristics.

Characteristic	NP-SLE (n=16)	SLE (n=18)	Controls (n=24)
Females, n (%)	15 (94)	12 (67)	10 (42)
Age at death in years, mean (SD)	44 (14)	46 (19)	47 (17)
Neuropsychiatric syndromes (ACR 99 definitions)*			
Cerebrovascular disease, n (%)	11 (69)	2 (11)	0 (0)
Movement disorder, n (%)	2 (13)	0 (0)	0 (0)
Seizures and seizure disorders, n (%)	1 (6)	0 (0)	0 (0)
Acute confusional state, n (%)	2 (13)	1 (6)	0 (0)
Cognitive dysfunction, n (%)	1 (6)	0 (0)	0 (0)
Psychosis, n (%)	1 (6)	0 (0)	0 (0)
No neuropsychiatric symptoms, n (%)	0 (0)	15 (83)	24 (100)
Primary versus secondary NP-SLE			
Primary NP-SLE, n (%)	16 (100)	0 (0)	0 (0)
Secondary NP-SLE, n (%)	0 (0)	3 (17)	0 (0)
Neurologic infection, n (%)	3 (19)	5 (28)	0 (0)
Brain mass in grams, mean (SD)	1308 (177)	1290 (137)	1437 (171)

* Eleven NP-SLE patients had one syndrome, four patients had two syndromes, and one patient had three syndromes.

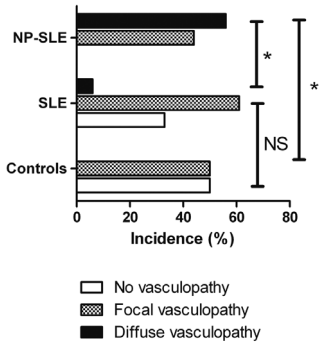
Figure 1 Histopathologic lesions in NP-SLE, SLE, and controls.



Frequency of the indicated lesions in brain tissue of SLE patients (n=18), NP-SLE patients (n=16), and control subjects (n=24). Microthrombi, microinfarctions, macroinfarctions, and vasculitis were present more often in the brains of patients in the NP-SLE group compared to those of patients in the SLE group (*, P<0.05 versus SLE). None of the seven parameters were present in the brains of control patients.

The presence of vasculopathy – and its distribution in either a focal or diffuse pattern – differed between the three groups based on linear-by-linear association ($P<0.001$; **Figure 2**).

Figure 2 Vasculopathy in NP-SLE, SLE, and controls.



Results of semi-quantitative scoring of vasculopathy in brain tissues of SLE patients (n=18), NP-SLE patients (n=16), and control subjects (n=24). Diffuse vasculopathy was present more often in the NP-SLE brains than in SLE and control brains. * $P<0.05$.

Vasculitis was found in six patients (five NP-SLE, one SLE; $P<0.050$). Each NP-SLE patient with vasculitis also had vasculopathy (four diffuse, one focal). Vasculitis was associated with cerebrovascular disease in four NP-SLE patients and with acute confusional state in one NP-SLE patient. The patient from the SLE group with vasculitis had a severe mycotic infection associated with immunosuppressive therapy.

Typical examples of vasculopathy and cerebral microthrombi are shown in **Figure 3A** and **3B**, respectively.

Correlations between neuropathological findings in patients with SLE and NP-SLE

In patients with SLE (NP-SLE and SLE), vasculopathy was correlated with macroinfarction ($\rho=0.43$, $P=0.012$) and microthrombi ($\rho=0.57$, $P<0.001$). Furthermore, microthrombi were correlated with microinfarction ($r=0.54$, $P=0.001$) and microbleeds ($r=0.40$, $P=0.018$). Large haemorrhage was correlated with macroinfarction ($r=0.35$, $P=0.042$) and vasculitis ($r=0.38$, $P=0.027$), and microinfarction was correlated with microbleeds ($r=0.46$, $P=0.006$).

Immunohistochemistry

Figure 3C-J shows typical examples of immunohistochemical staining with C1q, C4d, MBL, and C5b-9 in patients and controls. C1q, C4d, and C5b-9 were observed on endothelial cells of small vessels (**Figure 3D, F, J**). **Table 2** shows C1q, C4d, and C5b-9 staining patterns in patients and controls. Additionally, C5b-9 was observed on cells with the morphology of glial cells (**Figure 3I**). Cellular staining of C5b-9 was not different between patients and

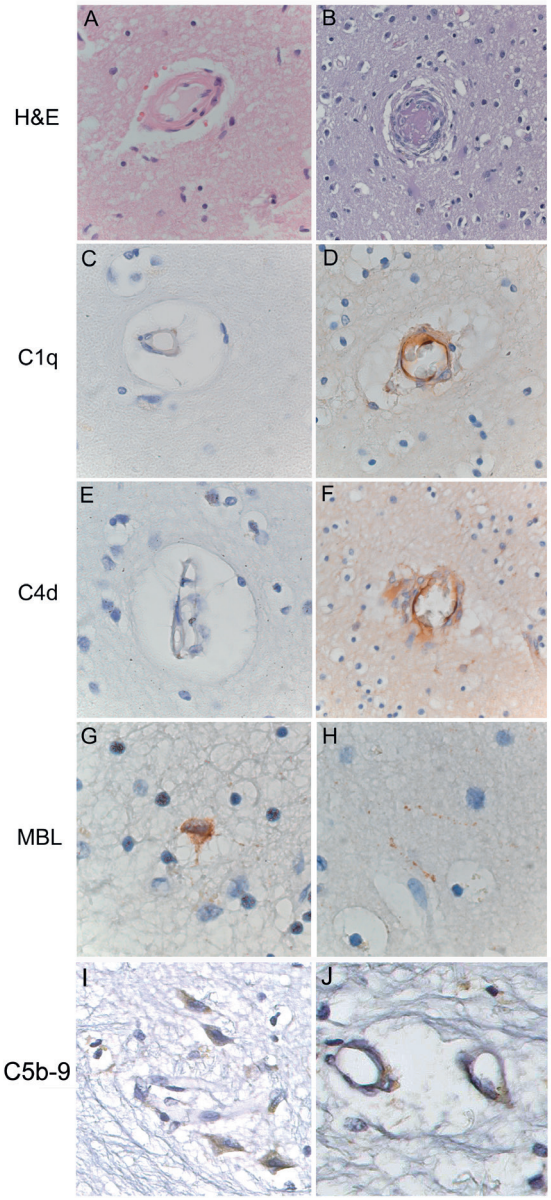
controls (data not shown). MBL deposits did not stain vessels; thus, co-localisation between MBL and C1q or C4d did not occur. Instead, MBL-positive staining was observed on cells resembling astrocytes. These MBL-positive cells (**Figure 3G–H**) were detected both in patients and controls (no difference between the groups; data not shown).

Table 2 Vascular C1q, C4d, and C5b-9 staining in patients and controls.

Staining pattern	NP-SLE (n=16)	SLE (n=18)	Controls (n=24)	*P**
No C1q staining	0 (0)	1 (6)	15 (63)	
Focal C1q staining	11 (69)	10 (55)	9 (37)	
Diffuse C1q staining	5 (31)	7 (39)	0 (0)	<0.001
No C4d staining	2 (12)	2 (11)	18 (75)	
Focal C4d staining	11 (69)	14 (78)	6 (25)	
Diffuse C4d staining	3 (19)	2 (11)	0 (0)	<0.001
No C5b-9 staining	3 (19)	3 (17)	20 (83)	
Focal C5b-9 staining	9 (56)	12 (67)	4 (17)	
Diffuse C5b-9 staining	4 (25)	3 (17)	0 (0)	<0.001

All values are given as n (%). C1q: NP-SLE vs. controls $P<0.001$; SLE vs. controls $P<0.001$; NP-SLE vs. SLE $P=0.531$.C4d: NP-SLE vs. controls $P<0.001$; SLE vs. controls $P<0.001$; NP-SLE vs. SLE $P=0.801$. C5b-9: NP-SLE vs. controls $P<0.001$; SLE vs. controls $P<0.001$; NP-SLE vs. SLE $P=0.796$. *Chi-square test.

Figure 3 Examples of histopathologic lesions and immunohistochemical staining patterns.



A, B, D, F, H, I, J: staining patterns in an NP-SLE case. C, E, G: staining patterns in a control case. Stainings: haematoxylin and eosin (H&E, A–B), C1q (C–D), C4d (E–F), mannose-binding lectin (MBL, G–H), and C5b-9 (I–J). Magnification: 40X. A: vasculopathy (thickening of the vessel wall, no inflammatory infiltrate). B: cerebral microthrombus. C–D: negative and positive C1q staining, respectively. E–F: negative and positive C4d staining, respectively. C1q showed a linear pattern of intravascular deposits (D), similar to C4d (F). G–I: positive glial cell staining. J: C5b-9 staining showing a linear pattern of intravascular deposits.

Complement deposition in relation to neuropathological findings in patients with SLE and NP-SLE

We investigated the relationship between C1q, C4d, C5b-9, and the presence of microthrombi, microinfarctions, macroinfarctions, vasculitis, and vasculopathy. Only the presence of microthrombi was associated with C4d and C5b-9 staining (P=0.047 and P=0.020, respectively). Every patient with microthrombi also had either focal or diffuse C4d and C5b-9 staining. C4d and C5b-9 were strongly associated with microthrombi within the NP-SLE group (linear-by-linear association: P=0.024 and P=0.008, respectively).

Relationship between C4d, C1q, and C5b-9 in SLE and NP-SLE

To gain additional insight into the cascade of events in classical complement activation, the staining patterns of C1q, C4d, and C5b-9 were related to one another in the SLE and NP-SLE patient groups. In general, diffuse C1q staining was present more often than diffuse C5b-9 and C4d staining (35%, 21%, and 15%, respectively). Twenty-five of 34 (74%) patients with SLE and/or NP-SLE had concurrent positive (either focal or diffuse) vascular staining of C1q, C4d, and C5b-9. Twenty-nine of the 30 patients with positive C4d staining (either focal or diffuse) had concurrent C1q deposits and 25 had concurrent C5b-9 deposits (an overlap of 97% and 83%, respectively). Conversely, 29 of the 33 patients with positive C1q staining (either focal or diffuse) had concurrent C4d, and 28 had concurrent C5b-9 staining (an overlap of 89% and 85%, respectively). Of the 28 patients with positive C5b-9 staining, 25 (89%) had concurrent C1q and C4d staining. **Appendix 6.3** shows that the distributions of C1q, C4d, and C5b-9 were quite similar in both the SLE and NP-SLE group.

Post-mortem ex vivo MRI and correlations with histopathology and immunohistochemistry

The results of post-mortem ex vivo MRI scans and histopathology sections of two NP-SLE patients and one SLE patient are shown in **Figure 4**.

Patient 1: Post-mortem ex vivo MRI

MRI revealed extensive confluent periventricular and deep white matter lesions with notable sparing of U-fibres (**Figure 4A–B**). Furthermore, central lacunae in the deep white matter suggested tissue loss consistent with lacunar infarction. White matter lesions around confluent white matter lesions and adjacent to deep white matter lesions were perivascular in distribution.

Patient 1: Histopathology

Sections taken from both the deep and periventricular white matter lesions revealed areas of recent and less recent microinfarctions and macroinfarctions. In one deep white matter lesion, multiple microthrombi (**Figure 4C–D**) were identified. In all sections, prominent vasculopathy in both grey and white matter was found (**Figure 4E**). Vasculopathy was present within white matter hyperintensities and infarcted areas, as well as in normal-appearing white and grey matter. Furthermore, vast areas of atrophied cortex – particularly in the proximity of infarctions – had laminar necrosis (**Figure 4F**).

Patient 2: Post-mortem ex vivo MRI

MRI revealed normal grey and white matter differentiation (**Figure 4G–H**). Several linear hyperintensities were characteristic of normal Virchow-Robin spaces (**Figure 4G**). Unlike Patient 1, the white matter in Patient 2 was homogeneous, and cortical thickness was normal.

Patient 2: Histopathology

Because no abnormalities were identified on MRI, sections were obtained from several cortical areas. Each of these revealed diffuse vasculopathy (**Figure 4I–J**). Furthermore, lymphocytes had invaded vascular walls of several veins and venules, reflected by the presence of fragmented nuclei and fibrinoid material (**Figure 4K**). No intravascular microthrombi, infarctions, or gliosis were identified.

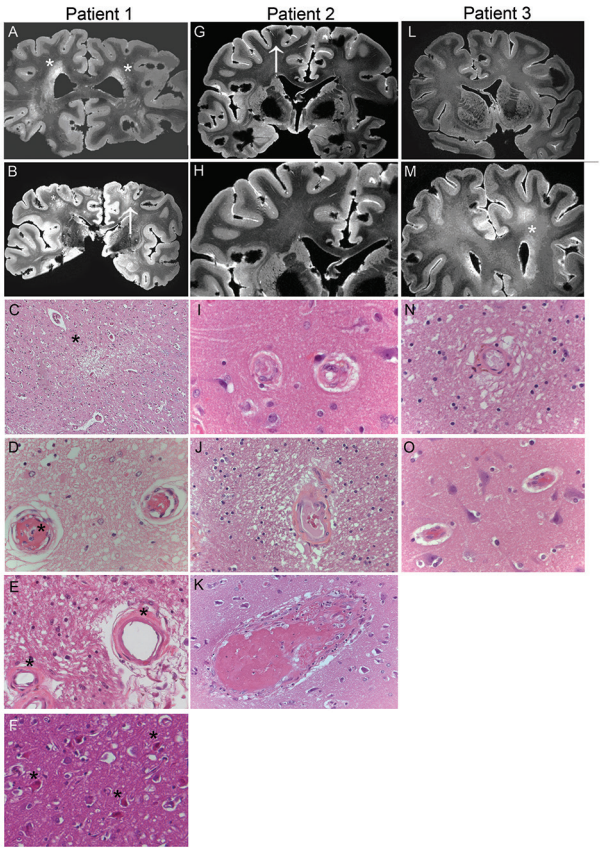
Patient 3: Post-mortem ex vivo MRI

MRI revealed a prominent Virchow-Robin space (**Figure 4L**). In addition, a linear perivascular white matter hyperintensity was present in the internal capsule. Another white matter lesion was identified in the frontal white matter (**Figure 4M**).

Patient 3: Histopathology

Sections taken from the frontal white matter lesion revealed focal vasculopathy. No other abnormalities were identified. Vasculopathy was present in a similar focal distribution pattern throughout all sections (**Figure 4N–O**).

Figure 4 Post-mortem 7-Tesla MRI in relation to histopathologic findings in NP-SLE and SLE patients.



Patients 1 and 2 were NP-SLE patients, patient 3 had SLE without neuropsychiatric symptoms. A: confluent periventricular and deep white matter lesions*. B: deep white matter lesions with sparing of U-fibres*; perivascular white matter lesions (arrow). C–F: sections from deep and periventricular white matter lesions. C: microinfarction*. D: microthrombi* in vicinity of white matter lesions. E: vasculopathy*. F: laminar necrosis*. G–H: normal grey/white matter with linear hyperintensities (normal Virchow-Robin spaces; G, arrow). I–K: sections from normal-appearing cortex. I–J: vasculopathy. K: venous vasculitis. L: prominent Virchow-Robin space and normal grey/white matter. M: frontal white matter lesion*. N: vasculopathy in section from M*. O: vasculopathy in section from normal-appearing area on MRI.

DISCUSSION

In this study, we described the injury in brains of patients with NP-SLE as compared to SLE and control cases. Furthermore, we studied brain injury in NP-SLE in relation to classical complement deposition. We were able to identify a number of histopathologic lesions that were specific to NP-SLE, and could thereby be linked to clinical NP-SLE manifestations as defined by the ACR.[2] Compared to SLE patients, NP-SLE patients had significantly more microinfarction, macroinfarction, vasculitis, and microthrombi, though in controls these and other neuropathological abnormalities were absent. Also, diffuse vasculopathy occurred significantly more often in NP-SLE than in SLE patients, whereas focal vasculopathy was found in all groups (NP-SLE, SLE, and controls). The common finding of vasculopathy and the absence of a relationship between vasculopathy and acute clinical NP-SLE are in accordance with previous findings.[15] Our data show that the injury and clinical manifestations of NP-SLE represent a continuum, ranging from relatively nonspecific lesions found in most SLE patients such as focal vasculopathy – to specific and even pathognomonic lesions in this setting, including diffuse vasculopathy and microthrombi associated with clinical syndromes defining NP-SLE.[2]

Our study is the first to demonstrate that deposits of C1q and C4d, both components of the classical complement pathway, as well as the terminal complement complex (C5b-9), are concurrently present in the cerebral vessels of patients with NP-SLE and SLE significantly more often than in controls. This constellation of staining patterns indicates that activation of the classical complement pathway proceeded to completion, as demonstrated by the formation of the terminal complement complex. The role of complement activation in the development of thrombosis and ischemia has been studied extensively outside the field of SLE, where complement has been linked to microthrombotic injury in antiphospholipid syndrome[19, 22] and thrombotic microangiopathy.[18] In these conditions, accumulation of antibodies in small vessels most likely leads to activation of the classical complement pathway, endothelial injury, and the subsequent formation of microthrombi.[18] Given that vasculopathy, microinfarction, macroinfarction, vasculitis, and microthrombi may all evoke vascular occlusion and could thereby result in clinical abnormalities, the question arises as to why some of these lesions were found to be more specific in our study for NP-SLE than other lesions. Our findings suggest that thromboischemic injury may occur in all SLE patients but that overt NP-SLE ensues only after a certain threshold of complement-mediated injury is reached. The finding that, of all vascular lesions, only microthrombi were correlated with C4d and C5b-9, suggests that microthrombi represent a more progressed stage of complement-mediated injury. Conceivably, microthrombi were also present in patients with subclinical NP-SLE – though in smaller quantities and would therefore be more readily missed in these cases due to sampling error.

Although cerebral vasculitis is generally considered to be a rare finding in NP-SLE, we identified five NP-SLE patients (31%) with vasculitis in our study. This relatively high incidence as compared to previous studies[12-15] may be explained by our clear distinction of SLE and NP-SLE cases. In four of the NP-SLE cases with vasculitis, vasculitis was associated with cerebrovascular disease. Interestingly, our neuropathological analyses revealed that vasculitis was correlated with large haemorrhage. Although according to the recommendations for the management of NP-SLE, cerebrovascular disease due to ischemic stroke and/or TIA comprises over 80% of cases and therefore does not require immunosuppressive therapy,[10] we showed that vasculitis may still be an underlying factor in a substantial number of cases. Our findings emphasise that immunosuppressive therapy may occasionally be indicated in cases with a component of vasculitis underlying cerebrovascular disease as a manifestation of NP-SLE.

Diamond *et al.* focused on the role of autoantibodies in the development of neuropsychiatric symptoms in a murine model and found that anti-double stranded DNA antibodies derived from human SLE patients can cross-react with N-methyl-D-aspartate receptors on neurons causing neuronal death by excitotoxicity and apoptosis.[28-30] However, in subsequent studies in which these antibodies were induced in mice, neuronal damage occurred solely when a breach in the blood-brain barrier was present.[31, 32] Our findings may indicate that continuously exposing the cerebral endothelium to autoantibodies can cause complement activation and endothelial injury in all SLE patients. Possibly, a second hit is required to develop overt clinical disease in the form of NP-SLE as defined by the ACR.[2] Infection, pregnancy, drug toxicity, and defects in complement regulation have all been described as triggering factors.[20, 21] Possibly, these evoke a breach in the blood-brain barrier together with the formation of C4d- and C5b-9-associated microthrombi as well as the other lesions we identified to be distinctive of NP-SLE. Since sera and cerebrospinal fluid (CSF) were not available from the patients in our study, it was not possible to further analyse the antibody profile in CSF in relation to the CSF/albumin ration as a possible indicator of blood-brain barrier integrity in concert with the assessment of thromboischemic injury in NP-SLE and SLE cases. Whether these or other factors affecting the integrity of the blood-brain barrier play a role in NP-SLE will be the subject of future studies.

Apart from the clinicopathologic paradox of impressive histopathologic cerebral lesions that are sometimes not accompanied by overt clinical NP-SLE, there is also a clinicoradiological paradox. In contrast to the clinicopathologic paradox, the latter is defined by the absence of abnormalities on conventional MRI in patients with over clinical NP-SLE, and this hampers assessment of the extent of cerebral injury in NP-SLE patients.[11] We investigated whether microvascular and thromboischemic injury could be detected clinically by analysing three whole brains of SLE patients using 7-Tesla MRI. High-field MRI can provide images at higher spatial resolution, resulting in more detailed information regarding microvascular injury compared to conventional MRI. Interestingly, for these brains (two NP-SLE, one SLE), even high-field MRI was unable to detect most small-vessel injury that was visible

histopathologically. The microvascular injury that could be detected was most prevalent in the vicinity of white matter hyperintensities. On the other hand, high-field MRI would detect nonspecific lesions more readily than conventional MRI.[33] White matter hyperintensities by conventional MRI may be found in 20 percent of the general population younger than age 50, and in 90 percent of people older than age 70.[33] Furthermore, white matter lesions may be observed in SLE patients who do not have neuropsychiatric symptoms. Indeed, white matter lesions were detected by 7-Tesla MRI in one SLE patient without NP-SLE in our study, which lesions corresponded to focal vasculopathy. Thus, although white matter hyperintensities may indicate an initial phase of vascular damage in some SLE patients, they should generally be considered nonspecific. Probably, more sensitive as well as more specific imaging tools are required for the diagnosis of NP-SLE. Future studies to determine the feasibility of, for instance, quantitative techniques such as volumetric magnetisation transfer imaging (MTI)[34, 35] to detect specific lesions in SLE and NP-SLE patients are called for, as brain biopsies remain unattainable in routine clinical practice.

Concluding, NP-SLE is a poorly understood manifestation of SLE, associated with significant morbidity and mortality. NP-SLE is usually treated with aggressive immunosuppression, which can be beneficial in some – but certainly not all – NP-SLE patients.[10, 36] Our finding that complement activation is present in both SLE and NP-SLE suggests that this mechanism may contribute to the development of injury. It remains to be elucidated whether the clinical manifestations of NP-SLE following injury are due to a thromboischemic pathomechanism, due to a breach in the blood-brain barrier facilitating antibody-mediated neuroinflammation, or due to a combination of both. Importantly, our novel finding of complement in association with injury in SLE and NP-SLE proposes the complement system as a promising new target in the treatment of NP-SLE. The finding that C5b-9 deposits were present in 82% of patients with SLE and NP-SLE in our study, suggests that at least this proportion of patients with NP-SLE – and perhaps also patients with SLE – may benefit from treatment with the terminal complement inhibitor eculizumab.[37-42] The efficacy of such treatment in NP-SLE and SLE remains to be investigated in future studies.

ACKNOWLEDGEMENTS

We thank Mariel Casparie and Lucy Overbeek from the PALGA network for allowing us to access the nationwide database of pathology laboratories. We also thank all pathologists who participated in this study by sending us cases from their labs and the clinicians who provided us clinical data (dr. R. Luijten and dr. J. Tekstra, UMCU, Utrecht; prof. dr. A.E. Voskuijl, VUmc, Amsterdam; dr. K. Ronday, HagaZiekenhuis, The Hague; dr. A. Spoorenberg, UMCG, Groningen; dr. P.D.M. de Buck, MCH, The Hague). Furthermore, we thank Corry Welling and Ingrid Hegeman for providing excellent technical assistance. Finally, we are grateful to Professor M.R. Daha and Professor L.A van Es for providing constructive feedback on the manuscript.

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