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Neuroimmunological and clinical studies in Neuropsychiatric Lupus Erythematosus (NPSLE)

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To Ida and Kjell

ABSTRACT

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with many organ manifestations and characteristic immunological abnormities including a large set of autoantibodies with affinity for nuclear and membrane components. Autoantibodies form immune complexes, which cause inflammation and tissue damage in affected organs. Neuropsychiatric symptoms in SLE (NPSLE) are common, but clinically we lack a systemic strategy/protocol to follow-up or investigate these patients. Symptoms are heterogeneous, including both central –and peripheral nervous system manifestations. Several cytokines and biomarkers as well as risk factors for NPSLE have been associated with specific manifestations. Especially symptoms of central nervous system origin often contribute to serious clinical outcomes.

The overall aim of this thesis was to provide a deeper understanding of NPSLE regarding clinical manifestations, to investigate the performance of potential new biomarkers and possible consequences that may be result from treatment with several immunosuppressive medications. Furthermore, to investigate two of the most common symptoms, stroke and seizures/epilepsy in SLE.

In study I, we analyzed whether the activity of the cytokines APRIL and BAFF was enhanced in the systemic and/or the intrathecal compartments in NPSLE patients, as compared to healthy and multiple sclerosis (MS) controls. We also studied the relationship between APRIL/BAFF and fatigue in NPSLE. Levels of APRIL in the intrathecal compartment were significant higher. Fatigue correlated to higher APRIL levels in NPSLE.

In study II, plasma and cerebrospinal fluid from NPSLE patients treated with several immunomodulatory medications and controls, were investigated for possible detectable John Cunningham virus (JCV) DNA occurrence. None of these samples showed detectable JCV levels.

In study III, we investigated the distribution of ischemic stroke subtypes in SLE patients, and classifying them according the system Trial of Org 10172 in Acute stroke Treatment (TOAST) with possible association with STAT4 and HLA-DRBI risk genotypes. The anti-phospholipid Syndrome (APS) and cardiovascular genes (CE) play important roles in ischemic stroke. Patients with stroke in APS/other determined etiology (OE) were also younger compared with other subgroups. The STAT4 genotype associated specifically to ischemic stroke in both APS/OE and CE subtypes.

In study IV, we report that the prevalence of classified seizures and epilepsy in SLE is 11.5%, and the majority of defined epilepsy occurs as a focal epilepsy. APS was more common in patients with epilepsy compared to epilepsy-free SLE patients with or without NPSLE. Cerebrovascular disease was highly significantly more common. In 50% of patients with epilepsy no etiology other than SLE was detected.

In conclusion, we have confirmed the importance of cytokines APRIL/BAFF in SLE, also detected in NPSLE in CSF. JCV DNA levels were not detectable in plasma or cerebrospinal fluid for the patients investigated in this study. Furthermore, patients with ischemic stroke seem to have association with APS or CE occurrence. Patients with defined epilepsy in SLE had mostly focal seizures. Cerebrovascular disease is common, and for about half the epilepsy cases we cannot determine any other etiology than autoimmune disease, SLE. Collectively, these findings support the notion of a multifaceted involvement of the nervous system in SLE.

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LIST OF PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals:

I. **L Hopia,** M Thangarajh, M Khademi, A Laveskog, E Wallström, E Svenungsson, M Andersson. Cerebrospinal fluid levels of a proliferation-inducing ligand (APRIL) are increased in patients with neuropsychiatric systemic lupus erythematosus. Scand J Rheumatol (2011);40:363–372

II. E Iacobaeus, **L Hopia**, M Khademi, M Lundén, A-L Hammarin, E Svenungsson, M Andersson. Analysis of JC virus DNA in NPSLE patients treated with different immunomodulatory agents. Lupus (2013) 22, 307–31

III. **L Hopia**, A Laveskog, A Jönsen, D Leonard, J T Gustafsson, I Gunnarsson, A Zickert, G Nordmark, A A. Bengtsson, K Elvin, L Padyukov, J K. Sandling, A-C Syvänen, L Rönnblom, M Andersson, E Svenungsson. Ischemic stroke in Systemic Lupus Erythematosus-Distribution of sub-types and association with *STAT4* and *HLA-DRB1* genes. Manuscript.

IV. **L Hopia**, M Andersson, E Svenungsson, M Khademi, F Piehl, T Tomson. Epilepsy in Systemic lupus erythematosus: Prevalence and risk factors. Submitted.

CONTENTS

LIST OF ABBREVIATIONS

Ab	Antibody
ACR	American College of Rheumatology
ANA	Antinuclear Antibodies
Anti-dsDNA	Antibodies to double stranded DNA
Anti-La/SSB	Anti-Sjögrens Syndrome antigen B Antibodies
Anti-Ro/SSA	Anti-Sjögrens Syndrome antigen A Antibodies
Anti-RNP	Anti-Ribonuclearprotein Antibodies
Anti-ribo P	Anti-Ribosomal P Antibodies
Anti-Sm	Anti-Smith Antibodies
aß$_2$GPI	Anti-ß$_2$ Glycoprotein I Antibodies
aCL	Cardiolipin Antibodies
aPL	Antiphospholipid Antibodies
APC	Antigen Presenting Cell
APS	Antiphospholipid Syndrome
APRIL	A-proliferation-Inducing Ligand
BAFF	B-cell Activation Factor
BBB	Blood Brain Barrier
Bly S	B lymphocyte Surface
C	Complement component
CE	Cardio-embolism
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computer tomography
EEG	Electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
EP	Epilepsy
FSS	Fatigue Severity Scale
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HLA	Human leucocyte antigen
IC	Immune complex
IFN	Interferon
Ig	Immunoglobulin
IHD	Ischemic heart disease
IL	Interleukin
ILEA	International League against Epilepsy
IRF	Interferon regulatory factor
JCV	John Cunningham virus
LA	Lupus anticoagulant
LAA	Large artery atherosclerosis
LE	Lupus erythematosus cell
MAC	Membrane-attack complex

MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MS	Multiple sclerosis
NMDA	N-methyl D-aspartate
NK	Natural killer
NP	Neuropsychiatric
NPSLE	Neuropsychiatric Systemic Lupus Erythematosus
OE	Other determined etiology (TOAST)
OND	Other neurological diseases
PB	Peripheral Blood
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PET	Positron emission tomography
PML	Progressive Multifocal Leukoencephalopathy
PNS	Peripheral nervous system
PRES	Posterior reversible encephalopathy syndrome
RR-MS	Relapsing-remitting MS
SAO	Small artery occlusion
SLE	Systemic Lupus Erythematosus
SLICC	Systemic Lupus International Collaborating Clinics
SNP	Single Nucleotide Polymorphism
SSP PCR	Sequence-specific primer PCR
STAT4	Signal transducer and activator of transcription 4
TACI	Transmembrane activator
TGFß	Transforming growth factor-ß
TIA	Transitory ischemic attack
TNF	Tumor Necrosis Factor
TOAST	Trial of Org 10172 in Acute Stroke Treatment
UE	Undetermined etiology (TOAST)
WHO	World Health Organization

1. SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

1.1. BACKGROUND

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with several organ manifestations and characteristic immunological abnormalities including a large set of autoantibodies (1-4). SLE mainly affects females with peak incidence between the ages of 15 to 40 and a male-to-female ratio of approximately 1: 9. However, SLE can also be diagnosed in young children and in the older population. The worldwide prevalence is estimated to be 3-207/100 000 and in Sweden 46-85/100 000 (5-7). The prevalence varies with race, ethnicity and socioeconomic status (5, 8-9). In order to classify as SLE the patient needs to fulfil four or more of the eleven criteria, based on the 1982 American College of Rheumatology (ACR) revised criteria for classification (10) that include 9 clinical and 2 immunological criteria. However, these criteria are made to classify patients in clinical studies. There are presently no diagnostic criteria and thus doctors can diagnose SLE even if the criteria are not fulfilled.

Neurological manifestations and neuropsychiatric symptoms are common and occur in approximately 50-90%, depending on definition. In general, neurological presentations are very heterogeneous and they include 19 Neuropsychiatric Lupus Erythematosus (NPSLE) syndromes as defined by the ACR. These syndromes affect both the central (CNS) and peripheral nervous systems (PNS) and they also include psychiatric manifestations (11, 12). One of the original 11 ACR classification criteria is neurological and includes the symptoms epilepsy or psychosis.

In 2012 the Systemic Lupus International Collaborating Clinics (SLICC) group proposed new classification criteria for SLE. These criteria have been extended with regard to NPSLE symptoms and include mononeuritis multiplex, myelitis, peripheral or cranial neuropathy and acute confusional states in addition to seizures and psychosis (13). Involvement of the central nervous system (CNS) is generally associated with poor prognosis in SLE (14-16).

1.2. CLINICAL FEATURES AND CLASSIFICATION OF SLE

The clinical manifestations are heterogeneous and presentations may differ during the course of SLE. In general, the disease course can be described as relapsing-remitting, chronic active or as a quiescent chronic active condition (17). The most frequently affected organs at any time are joints (arthritis/arthralgia) and skin with photosensitivity, discoid lupus or malar rash. Further, 30-40% are affected with nephritis, 40% with serositis presenting either as pericarditis or pleuritis. Symptoms of SLE can be mild and limited to only a few organs, but disease flares can also be aggressive with involvement of several organs simultaneously.

According to the revised criteria for SLE by Tan 1982, 11 criteria are used to define SLE. There are 9 clinical or routine laboratory criteria: malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurological disorder or hematologic disorder, immunologic disorder or detectable antinuclear antibodies (ANA). Additionally, there are two immunological criteria including: 1) antibodies to double stranded DNA (anti-dsDNA) or anti-Smith (anti-Sm) antibodies, positive lupus erythematosus (LE) cell staining or 2) an abnormal titer of ANA by immunofluorescence. A definite SLE classification can thus be established if the patient has presented at least four of the eleven criteria. Table 1. Antibodies to double stranded DNA

Severe fatigue is a common and disabling symptom in SLE. It has also been reported by patients with chronic inflammatory rheumatic disease, as well as for patients with multiple sclerosis (MS) (18, 19). However, it is not defined by the ACR as a clinical feature in SLE or in NPSLE.

Table 1. The 1982 revised criteria for classification of systemic lupus erythematosus*

Criteria	Definition
1. Malar rash	**Fixed erythema**, flat or raised, **over the malar eminences**, tending to spare the nasolabial folds
2. Discoid rash	**Erythematous raised patches with adherent keratotic scaling** and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	**Oral or nasopharyngeal ulceration**, usually painless, observed by a physician
5. Arthritis	**Non-erosive arthritis involving 2 or more peripheral joints**, characterized by tenderness, swelling, or effusion
6. Serositis	**Pleuritis** - convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion or **Pericarditis** - documented by ECG or rub or evidence of pericardial effusion
7. Renal disorder	Persistent **proteinuria greater than 0.5 grams per day** or greater than 3+ if quantitation not performed. Cellular casts - may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurological disorder	**Seizures** - in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance **Psychosis** - in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance
9. Hematologic disorder	**Hemolytic anemia**-with reticulocytosis. **Leukopenia**-less than 4,000/mm^3 total on 2 or more occasions OR **Lymphopenia**-less than 1 ,500/mm^3 on 2 or more occasions or **Thrombocytopenia**-less than 100,000/mm^3 in the absence of offending drugs
10. Immunologic disorder	**Positive LE cell preparation Anti-DNA**: antibody to native DNA in abnormal titer Anti-Sm: **presence of antibody to Sm nuclear** antigen False positive serologic test for syphilis known to be positive for at least 6 months and confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test An abnormal titer of antinuclear antibody
11. Antinuclear antibody (ANA)	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with “drug-induced lupus” syndrome

** The proposed classification is based on 11 criteria. For the purpose of identifying patients in clinical studies, a person shall be said to have systemic lupus erythematosus **if any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation (10).***

1.3. AUTOANTIBODIES IN SLE

Autoantibodies play a central role in SLE, which has been proposed to be the autoimmune condition with the largest number (>100) of detectable autoantibodies. These include autoantibodies that target nuclear antigens, cytoplasmic antigens, cell membrane antigens, phospholipid-associated antigens, blood cells, endothelial cells, nervous system antigens, plasma proteins, matrix proteins and miscellaneous other antigens. The targets of these autoantibodies, the autoantigen IC properties, autoantibody frequencies, as well as clinical associations, and correlation with disease activity have previously been described (20, 21).

Anti-nuclear antibodies (ANA) are present in 95-99% of SLE patients and antibodies against double-stranded DNA (anti-dsDNA) are present at any time in about 50-70%, and they have been shown to increase just prior to SLE onset and they are often positive in nephritis patients (22). Certain autoantibodies have been connected with clinical features of SLE. For example, anti-ribosomal P (anti-ribo P) antibodies have been linked with psychosis (23) and anti-Smith (anti-Sm) antibodies with milder renal and CNS disease, as well as with relapse of SLE. Other antibodies such as Sjögrens syndrome (Antigen- A and B) -associated anti- Ro (SSA), and anti-La/SSB are associated with secondary Sjögren´s syndrome, lymphopenia and a milder disease course while anti-C1q are associated with nephritis. Furthermore anti-histone antibodies have been reported in some cases of drug-induced lupus (23).

1.3.1 Antiphospholipid antibodies (aPL) and APS syndrome

The presence of aPL, a heterogeneous group of autoantibodies are associated with increased risks of thrombotic events in SLE and in the general population. These antibodies are thus more commonly detected in patients with a variety of vascular events. A large international study based on 120 full-text papers, though the majority were published before 1 reported the following frequencies: 13.5% in patients with stroke, 11% in myocardial infarction (MI), 9.5% in deep venous thrombosis (DVT) and 6% among women presenting with pregnancy morbidity (24).

The antiphospholipid antibody syndrome (APS) is defined by the presence of antibodies against cardiolipin of IgG or IgM isotypes (IgG aCL, IgM aCL) or antibodies

against phospholipid binding cofactor ß$_2$glycoprotein-I (GPI) of IgG or IgM isotypes (IgG anti-ß$_2$GPI, IgM anti-ß$_2$GPI) or positivity in the functional lupus anticoagulant (LA) test. One or more thrombotic (venous, arterial-or small vessel) event in any tissue or organ, and/or pregnancy-related morbidity are needed (24-26) to classify for the clinical APS criterion. At least one clinical criterion as well as a minimum of two positive tests at least 12 weeks apart are required for a confirmed APS diagnosis (27).

APS was first described in patients with SLE, i.e. secondary APS, but it is now regarded as a separate entity. When APS occurs in the absence of other autoimmune disorders it is referred to as (primary APS). The definition is the same for primary and secondary APS.

1.4. THE IMMUNE SYSTEM

A vital function of the immune system is to defend the body from invasion by bacteria, viruses and parasites, but it is also involved in other important functions such as repair after tissue injury and cancer cell surveillance. At a very basic level, the immune system can be subdivided into two parts; natural/innate and specific/adaptive immunity.

The innate system consists of mechanical barriers such as skin and mucosa, phagocytic cells of the myelomonocytic lineage (macrophages, dendritic cell, neutrophils and natural killer (NK) cells) and serum constituents such as complement proteins and cytokines. The innate immune system acts by recruiting immune cells to sites of infection or damage and producing cytokines and other mediators of inflammation. Activation of antigen presenting cells (APCs) and the complement cascade will also act as a bridge to the adaptive arm of the immune system.

The adaptive immune system consists mainly of T and B lymphocytes, which represent the two major subdivisions, humoral and cell-mediated immunity, respectively. The humoral part consists of B cells that have antigen presenting properties and produce antibodies. Further maturation of B cells will lead to long-lived plasma cells, which are the main source of antibodies/immunoglobulin (Ig) molecules. The cell-mediated arm comprises T cells with cytotoxic or regulatory capacity.

T lymphocytes can be further divided into major subsets T-helper ($CD4^+$) and cytotoxic ($CD8^+$) cells. However, additional T cell populations include also other types of

cells, such as regulatory T cells that are important for regulation of adaptive immune responses. Based on the types of cytokines they produce, $CD4^+$ T-helper cells can be classified into additional phenotypes including T helper 1 (Th1) and T helper 2 (Th2), T helper 17 (Th17) and T regulatory cells (Treg). Differentiation into either of these two phenotypes leads to preferential secretion of certain signature cytokines. Th1 cells secrete interferon-γ (IFN-γ), interleukin 2 (IL-2), interleukin 12 (IL-12), and tumor necrosis factor-α (TNF-α), which promote cell-mediated responses. Interleukins 4, 5, 9 and 10 (IL-4, 5, 9, 10) instead are secreted by Th-2 cells and promote humoral or allergic responses. Th17 cells secrete interleukins-17, -21 and -22 (IL-17, -21, -22) and are believed to be of importance for mediating tissue inflammation including autoimmune disease. Tregs play an important role for mediation of immune tolerance and controlling immune responses so that they do not go out of control, which is mediated by secretion of cytokines such as interleukin -10 (IL-10) and transforming growth factor-ß (TGFß).

The adaptive immune system is different from the innate immune system in that it can learn to recognize (adapt) and mount a very strong immune response against distinct antigens. Unfortunately, antigen recognition may cross-react with self-peptides or other altered self-molecules and thus create the basis for autoimmune diseases.

1.4.1. The complement system

The complement system has a protective function in our immune system. It consists of plasma proteins and the activation of one component leads to a cascade of reactions. The classical, mannose-binding lectin and alternative complement pathways make up this system, each of them utilizing specific chemical functions. All lead to formations of enzymatic complexes (C3 and C5) and further reactions that attract white blood cells and form a cytotoxic membrane- attack complex (MAC).

The classical pathway is mainly interactive with complement component C1q and IgG and IgM antibodies. The complement system has many biological functions. It facilitates phagocytosis and clearance of immune complexes (ICs) and dying cells, and it also attracts inflammatory cells to sites of complement activation.

Typical findings in SLE are low serum concentrations of complement C1q, C3 and C4, mainly during flares (28). Furthermore, genetic C1q deficiency is a rare but strong risk factor for SLE. Individuals with genetic complement deficiencies, e.g. of C1q, C2 and C4, are

strongly predisposed to develop SLE (29). These findings may contribute to our understanding of up-regulation of the type I IFN system seen in SLE patients (28).

1.4.2. The immune system and SLE

In SLE a central immunologic disturbance is the excessive production of autoantibodies that induce damage via several mechanisms. Autoantibodies target self-molecules that are present in the nucleus, the cytoplasm and on cell surfaces. Antibodies together with their antigens and complement proteins form immune complexes, which are trapped, or possibly formed in tissues, where they can activate complement and cause inflammation and damage. The inflammatory state leads to further immune cell abnormalities and dysregulation that involves B cells, T cells and cells of the monocyte lineage. Both innate and adaptive immunity are highly involved.

Increased systemic inflammation can be detected in patients with SLE. However, classical measures of inflammations, such as C-reactive protein are not good markers of SLE disease activity. Rather, high levels of multiple pro-inflammatory cytokines in peripheral blood have been reported in active SLE disease.

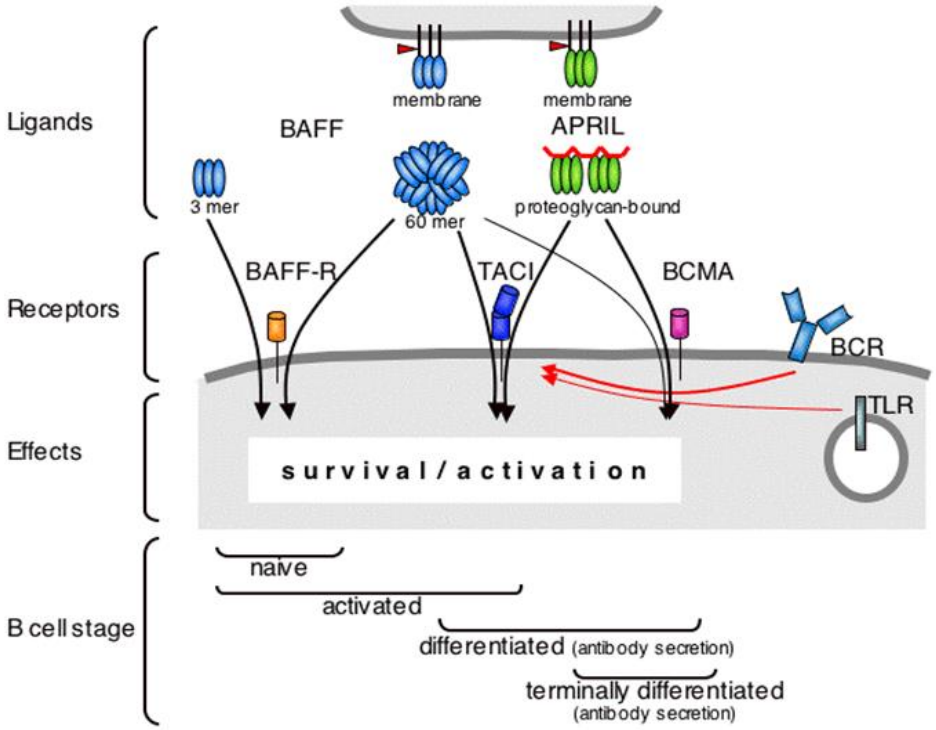
Type I interferons (IFN), including interferon-α (IFN- α) have been demonstrated to correlate both with disease activity and severity, and this is a key cytokine in lupus. Type I IFNs have also being associated with vascular disease in SLE (30-32). TNF-α is another cytokine that together with its soluble receptors 1 and 2 reflects disease activity (33,134). The complement system is also important in SLE, where peripheral blood levels of several complement proteins are depressed due to consumption during more active disease. This is especially common during active nephritis (29, 34).

1.4.3. B-cell efficiency- BAFF and APRIL

B cells require signals from several sources for their maturation from precursor cells and for further developing to effector cells. Two closely related cytokines belonging to the tumor necrosis factor (TNF) family are involved in various human autoimmune conditions (35). High levels of both B-cell activating factor (BAFF) and a-proliferation-inducing ligand (APRIL) have been demonstrated in patients with SLE and in other autoimmune conditions such as rheumatoid arthritis and Sjögren’s syndrome (36-38).

BAFF, also known as TALL-1, THANK, Bly S, zTNF4 and TNFSF13, and APRIL, also known as TRDL-1, are both linked to B-cell survival, Ig isotype switching, and B-cell antigen presentation (39-41). They are present in the circulation as soluble trimeric ligands, but BAFF can also be detected as a membrane-bound protein (40).
Both of these cytokines bind to two receptors, B-cell maturation antigen (BCMA) and transmembrane activator (TACI), BAFF also separately binds to BAFF-R, and thereby regulates B-cell survival. Figure 1.

Figure 1.



BAFF and APRIL were highlighted at the time of our study, since clinical trials of a new human anti-soluble BAFF monoclonal antibody, Belimimab, were ongoing. Efficacy in lupus patients has since been demonstrated. Belimimab is now registered for treatment of SLE and is has been shown to reduce both disease activity and fatigue (42, 43).

1.4.4. Genetics

More than 60 genetic variants have been linked to risk of SLE. The most strongly associated genetic region is the HLA region, located on chromosome 6, which contains many genes of importance for the immune system and for autoimmunity including those encoding for the major histocompatibility class I and class II chains. Some of the identified SLE susceptibility genes located elsewhere in the genome are linked to interferon signaling, such as interferon regulatory factor 5 (IRF5) and signal transducer and activator of transcription 4 (STAT4), both of which have been repeatedly confirmed to be associated with SLE (44-46). Additionally, although less common, persons with genetic deficiencies of complement proteins involved in the classical complement activation pathway are prone to develop SLE (29, 47).

2. CLINICAL SYMPTOMS IN NPSLE

Neurological symptoms in SLE are common and may have multiple causes. Of the 19 NPSLE case definitions by ACR (11) 12 are syndromes or manifestations from the CNS: aseptic meningitis, cerebrovascular disease, demyelinating syndrome, headache, movement disorder, myelopathy, seizure disorder, acute confusional state, anxiety disorder, cognitive dysfunction, mood disorder and psychosis, while 7 involve the PNS; Guillain-Barré, autonomic disorder, mononeuropathy, myasthenia gravis, cranial neuropathy, plexopathy and polyneuropathy. PNS symptoms are less common than CNS involvements. Table 2.

Involvement of the CNS in SLE is potentially severe, but still in most cases a treatable condition (48). Unfortunately, there is a lack of validated biomarkers for CNS involvement and neuroradiological findings may be non-specific. The fact that opportunistic infections, sometimes a consequence of immunosuppression, may mimic NPSLE symptoms further complicates the diagnostic work-up (49-53). Several cytokines have been suggested to be increased during fatigue in SLE, but studies so far have not provided evidence for association to disease activity or other markers of disease or inflammation (54).

Table 2. 19 NPSLE syndromes as defined by the American College of Rheumatology

Central Nervous System	***Peripheral Nervous System***
▪ Aseptic meningitis	▪ Acute inflammatory demyelinating polyradiculopathy (Guillain-Barré)
▪ Cerebrovascular disease	▪ Autonomic disorder
▪ Demyelinating syndrome	▪ Mononeuropathy, single or multiplex
▪ Headache (including migraine and idiopathic intracranial hypertension)	▪ Myasthenia gravis
▪ Movement disorder (chorea)	▪ Cranial neuropathy
▪ Myelopathy	▪ Plexopathy
▪ Seizure disorder	▪ Polyneuropathy
▪ Acute confusional state	
▪ Anxiety disorder	
▪ Cognitive dysfunction	
▪ Mood disorder	

Extensive efforts have been made to better understand the underlying pathological mechanisms for neurological symptoms in NPSLE, and several candidate biomarkers have

been suggested (55). However, many issues remain to be resolved and it is likely that several mechanistic pathways are involved and probably overlap. Neuropathological studies, by necessity performed in severe and dramatic clinical manifestations of CNS involvement, often demonstrate ischemic changes in small vessels such as cortical microinfarctions and a general vasculopathy with degenerative or proliferative changes generally (23). Reductions in cerebral and corpus callosum volumes and atrophy have been associated with disease duration and the history of CNS involvement and cognitive impairment. Furthermore, the total corticosteroid dose and the presence of aPL have not shown association to brain atrophy (56, 57). Macroscopic infarctions are less common. Embolism often arises from the heart, due to heart valve involvement, usually more discrete but sometimes in accordance with Libman-Sacks endocarditis (58-61). Both inflammatory infiltrates and destruction of blood vessel walls may occur, but necrosis indicative of vasculitis is rarely observed.

NPSLE is associated with systemic-and brain-specific antibodies (62). A recent meta-analysis of serum and CSF autoantibodies by Ho et al (51) reported that NPSLE patients, mainly those with CNS symptoms, tend to have higher levels of aPL including aCL and LA, as well as, anti-ribo P abs and anti-neuronal abs compared with SLE patients without neuropsychiatric symptoms. This was especially evident for patients with psychiatric conditions, cognitive impairment, cerebrovascular disease and seizures. Additionally, brain-specific abs such as anti-ganglioside, anti-glial fibrillary acidic protein, anti-lymphocytotoxic and anti-NMDA as well as anti-neuronal abs were mainly encountered in neuropsychiatric conditions. Neuropathy, psychosis and seizures have been reported to associate with anti-microtubule protein 2 antibodies leading to changes in neuron morphology (63).

One possible mechanism for vascular occlusion and injury by pathogenic antibodies is a disrupted blood brain barrier (64). Antibodies can mediate neurological injury by reacting against neuronal, astroglial or endothelial cells (23, 65-67). According to a more recent hypothesis, subtypes of N-methyl D-aspartate (NMDA) receptors may become targets for cross-reactive anti-DNA antibodies and the neuronal excitations, by way of excessive activation, may lead to neurocognitive disturbances and eventually to brain atrophy (67-70). aPL occur in 30-40% of SLE patients (71) and are associated with thrombotic and/or obstetric manifestations and seizures (30, 64, 72, 73). Furthermore, interferon-α (IFN-α) and other type I interferons have been demonstrated to correlate both with disease activity and severity and also have strong associations to vascular disease in SLE (22). Elevated levels of APRIL have also been reported in cerebrospinal fluid for SLE patients with neuropsychiatric symptoms as compared to controls (74).

Neuropsychiatric lupus erythematosus syndromes are often associated with global disease activity (75). Nonspecific cerebrospinal fluid (CSF) abnormalities are present in about 30% of patients, including elevated levels of monocytes, elevated protein levels or abnormalities in intrathecal production of immunoglobulins (76, 77). Levels of cytokines such as IL-6, -8 and -10 has been reported to be increased in the CSF of individuals with neurolupus compared with non-CNS lupus (78). The electroencephalogram (EEG) changes in NPSLE patients are often nonspecific generalized slowing (48).

Imaging with computed tomography (CT) may show evidence of cerebral infarction and hemorrhage, cortical micro-infarcts or vasculopathy and degenerative changes, and in chronic cases subcortical atrophy. Magnetic resonance imaging (MRI) is useful especially in patients with diffuse presentations. It reveals small infarcts in acute states, focal cerebral edema and detects myelitis better than CT. However, there are no MRI findings that are diagnostic of NPSLE (78, 79). Positron emission tomography (PET) has also been used to detect areas of disturbed cerebral circulation and metabolism (80).

Neuropsychiatric manifestations are common in SLE and may present in the context of general disease activity or as isolated events. Patients often present with mixed neurologic and psychiatric manifestations. The prevalence of NP manifestations according to ACR case definitions is variable. Accordingly several studies report that from 14% to over 90% of SLE patients presenting with neurological or psychiatric symptoms at some time during their illness (78). A recent large international, multicenter prospective study reported that 40.3% of the patients had at least one clinical NP event and 17.4% were diagnosed with multiple events (81). Different results may be due to technical variations between the studies, study designs and selections of the patients, study populations and how the original information was obtained. However, the most common NPSLE symptom reported are related to CNS manifestations. Most frequent are intractable headaches 24% (82, 83) including mostly tension-type and/or migraine, cluster headache or pseudo tumor cerebri. The second most common manifestation is cerebrovascular disease 14.5%, including transitory ischemic attack, ischemic stroke, subarachnoid/intracranial hemorrhage and sinus thrombosis. Mood disorders with major depressive episodes, depressive features and with manic features are common. Cognitive disorder have been reported to occur in about 11% and seizures in 8.3%. Psychosis as well as anxiety disorders are fairly common symptoms. Manifestations from PNS are seen more seldom, except polyneuropathy and cranial neuropathy (82). According to a Finnish study at least one NPSLE syndrome was identified in 91% of SLE patients (84). Cognitive dysfunction was present in 81%, followed by headache in 54% and mood disorder

in 43%. Headache, cognitive dysfunction and psychiatric disorders followed by seizures are also reported as the most common NPSLE syndromes in other studies (12, 85).

2.1. CEREBROVASCULAR DISEASE AND SLE

Stroke is defined by rapid onset of focal neurological deficit due to infarction or hemorrhagic vascular event lasting more than 24 hours, being the second most common cause of death worldwide and most common cause of neurological disability (86-89). A populations-based stroke register study in European countries reported an annual stroke incidences of 63-239/100 000 with a median age of 73 years, higher rates of stroke being reported in eastern and lower rates in southern European countries (90), with an incidence of 144/100 000 in Sweden (91).

Early risk of stroke following a transitory ischemic attack (TIA) is high; the 7-day risk is 8-12% and 30 day risk 18%. Traditional well-established risk factors are: age >55 years and male gender, hypertension, smoking, diabetes mellitus, hypercholesterolemia, atrial fibrillation and myocardial infarction.

A recent population-based meta-analysis by Holmqvist *et al.* (92) reported that individuals with SLE have a two-fold increased risk of ischemic stroke compared to the general population and that the relative risk of stroke was highest among individuals younger than 50 years of age. Another large meta-analysis performed with a wider clinical material of rheumatic diseases reported risk of any stroke to be particularly higher for patients with rheumatoid arthritis and systemic lupus erythematosus (93). In a large cross-sectional study from our group ischemic cerebrovascular disease occurred in 12% of Swedish SLE patients (44). The mean annual stroke incidence in another SLE cohort was 6.45/1000 patients where 90% had ischemic strokes and 10% hemorrhagic events. Furthermore, stroke occurred in 57% of SLE patients with a history of TIA (94, 95). High mortality 20-30% in SLE occurs due to cerebrovascular events.

2.1.1. Stroke subtypes according to TOAST

The Trial of Org 10172 in Acute Stroke Treatment (TOAST) (96) criteria is the most commonly used for pathogenic classification of stroke cases. It is designed to classify ischemic strokes, and is mainly based on etiology, being defined for use in a multicenter clinical trial setting, TIA being excluded. Five major categories of the TOAST classification are as follows: large-artery atherosclerosis (LAA); cardio-embolism (CE); small artery occlusion (SAO); stroke of other determined cause (OC); and stroke of undetermined cause (UE). The sub-type definitions are based on risk factor profiles, clinical features, and results of diagnostic tests (97).

A population-based prospective study for incidence, recurrence and long-time survival rates of ischemic stroke in European populations according to TOAST criteria, reported the following distributions of stroke sub-types: CE 27%, SAO 23%, LAA 13%, OE 2% and UE 35% (97, 98). Another previous prospective study among SLE patients reported that the LAA was most common sub-type, in 45%, SAO occurred in 39% and CE in 9% followed by 7% of UE (131).

2.1.1.1. Large artery atherosclerosis (LAA)

Patients in this category should have a history of large-artery atherosclerosis, supported by clinical and brain imaging findings. Typically findings for these patients include a significant (>50%) stenosis or occlusion of a major brain artery or branch cortical artery or in an extracranial artery, and history of transient ischemic attacks (TIAs) in the same vascular territory with symptoms of cerebral cortical impairment. Ischemic lesions located in cerebral cortex, cerebellum, brainstem or in subcortical areas should be greater than 1.5 cm in diameter as visualized by CT or MRI.

2.1.1.2. Cardio-embolism (CE)

The TOAST classification includes both high- and medium risk sources for cardio-embolism causes of an arterial occlusion and stroke. Clinical evidence of previous TIA or stroke in

more than one vascular territory or systemic embolism supports a clinical diagnosis of cardiogenic stroke. The patients have similar clinical and brain imaging findings as in the LAA group.

2.1.1.3. Small artery occlusion (SAO)/lacunar

Patients in this group should have a normal CT/MRI examination or a relevant brainstem or subcortical hemispheric lesion with a diameter of less than 1.5 cm. Clinical diagnosis is also supported if the patient has a history of diabetes mellitus or hypertension.

2.1.1.4. Acute stroke of other determined etiology (OE)

Non-atherosclerotic vasculopathies, hypercoagulable states and hematologic disorder are all represented in this category. According to TOAST this subgroup includes patients with rare causes of stroke. Patients should have clinical and CT or MRI findings of an acute ischemic stroke, regardless of the size or location. Diagnostic studies such as blood tests or arteriography should reveal one of these unusual causes of stroke. Cardiac sources of embolism and large artery atherosclerosis should be excluded. In our study we included all patients with APS and SLE, as a known multisystem disease and without evidence of these other conditions, in this sub-group.

2.1.1.5. Stroke of undetermined etiology (UE)

This category includes the cases of unclear etiology despite an extensive evaluation or insufficient evaluation. The patients with two or more potential causes of stroke, where the physician is unable to make a final diagnosis are included in this group.

2.2. EPILEPSY, SEIZURES AND NPSLE

Seizures are a well-recognized complication (85, 99) in SLE and are suggested to be a result of a combination of immune, vascular, metabolic and inflammatory mechanisms. Furthermore, the generalized seizures have been associated with elevated levels of IgG, oligoclonal bands, and increased cytokine production, also with enhanced titers of anti-neuronal antibodies (85, 100). Seizures have also been reported to be an early CNS manifestation (48, 101) in SLE and are in some studies also diagnosed as an isolated epilepsy (102). Variable frequencies and prevalence of seizure disorder are described, from 6–51% of adults and pediatric SLE patients (12, 82, 84, 103-106) and is reported to be more frequent in children. Furthermore, unprovoked seizure in MS and SLE in population-based case-control studies reported increased risks in both of these conditions (107).

2.2.1. Definition of epilepsy

Epilepsy has been defined as a condition characterized by recurrent (two or more) epileptic seizures, unprovoked by any immediate identified cause in the 1993 International League Against Epilepsy (ILAE) Commissions report (108). The new conceptual ILAE from 2005 defines epilepsy as a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological and social consequences of this condition (109).

As follow-up of this conceptual definition, the ILEA in 2014 published a report describing a practical clinical definition of epilepsy. In accordance to this, epilepsy is a disease of the brain that can be defined by any of the following conditions: (I) At least two unprovoked (or reflex) seizures occurring >24 h apart; (II) one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years; (III) diagnosis of an epilepsy syndrome (110, 111).

Important for an epilepsy diagnosis is the distinction between unprovoked and provoked (acute symptomatic) seizures. A seizure provoked by a transient factor acting on an otherwise normal brain to temporarily lower the seizure threshold does not qualify for epilepsy, even if recurrent. Epileptic seizures occurring in conjunction with an SLE flare or a

metabolic derangement, are hence considered to be acute symptomatic and do not count as epilepsy.

Epilepsy is considered to be resolved for individuals who either had an age-dependent epilepsy syndrome, but are now past the applicable age or who have remained seizure-free for the last 10 years and off anti-seizure medicines for at least the last 5 years. `Resolved´ is not necessarily identical to the conventional view of `remission´ or `cure´.

2.2.2. Definitions of seizures

The definition and classification of seizures differs from that of epilepsy. A seizure is a transient occurrence of signs and/or symptoms of epilepsy due to abnormal excessive or synchronous neuronal activity in the brain (109). The seizure presentations depend on the location of onset in the brain and the pattern of propagation. The seizures can further affect sensory, memory, cognition, or behavior. Somatosensory, auditory, visual, olfactory, gustatory and vestibular sensory and also more complex internal sensations that can be presented (109).

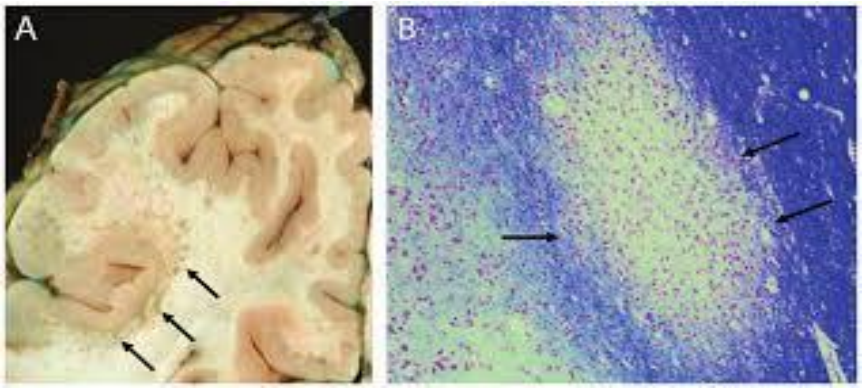
Seizures are classified as either focal or generalized, the former focal involving a localized part of the brain whereas generalized seizures rapidly involve the whole of both brain hemispheres from the onset. Secondary generalized describes the condition in which a focal seizure from a primary focus in some part of brain spreads into both hemispheres and becomes generalized. This classification does not describe the etiology, prognosis or pathophysiology for causes of seizures or epilepsy.

2.3. JC–VIRUS, PML and SLE

Progressive multifocal leukoencephalopathy (PML) is a rare but often fatal demyelinating disease of the CNS caused by the polyomavirus John Cunningham virus (JCV), with a mean survival time of 3-6 months (112). Due to immunosuppression the virus can reactivate and enter the brain and infect oligodendrocytes, causing a fulminate infection, demyelination, PML and further pathological destruction of the brain (113). (Figures 2 and 3). The primary infection occurs in up to 80% of the individuals, usually asymptomatic and after infection the virus tend to stay latent in the kidneys, bone marrow and lymphoid tissues and glial cells. Both T- and B cell effector functions are required to prevent viral reactivation (114).

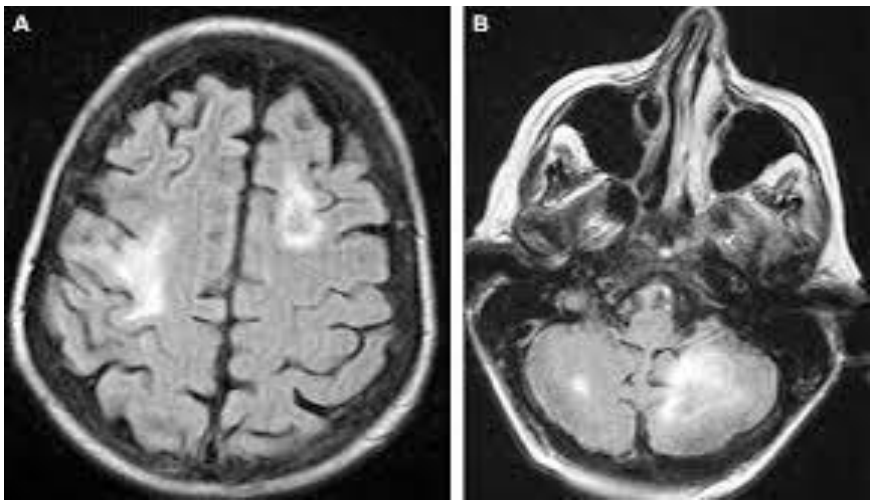
The incidence of PML in autoimmune diseases, including rheumatic diseases and mostly in patients with systemic lupus erythematosus has increased as a consequence of use of more potent immunosuppressive drugs such as biologics (rituximab, natalizumab, infliximab and efalizumab), methotrexate, azathioprine, cyclophosphamide and mycophenolate mofetil and even with a minimum of immunomodulatory treatments (115-119).

Figure 2.



Pathology of progressive multifocal leukoencephalopathy (PML). (113)
A. PML lesions affecting the superficial subcortical gray-white matter junction in the cerebral hemisphere. Multifocal punctate demyelinating lesions in subcortical white matter.
B. Microscopic demyelinated lesion in the white matter immediately subcortical. The cortex and neuronal cell bodies are in the left of the picture.

Figure 3.



Brain MR imaging of PML lesions associated with lupus and methotrexate overdose (119)

SLE/NPSLE is a complex condition with several immune aberrations. In our studies included in this thesis I have chosen to concentrate on some important clinical manifestations: stroke, epilepsy, PML, biomarkers and fatigue.

3. AIMS OF THE THESIS

The overall aim of this thesis was to provide a deeper understanding of NPSLE regarding clinical manifestations, potential biomarkers and pathophysiology.

The specific aims of this thesis were as follows:

1 To study whether APRIL/BAFF activity is enhanced in the systemic and/or the intrathecal compartments in neuropsychiatric SLE (NPSLE) patients compared to healthy controls, and to study the relationship between APRIL/BAFF and fatigue in neuropsychiatric SLE

2 To analyze whether JCV DNA can be detected in plasma or cerebrospinal fluid (CSF) in a large group of patients with NPSLE treated with immunomodulatory medication.

3 To investigate the distribution of ischemic stroke subtypes in SLE patients, classified according the TOAST system (Trial of Org 10172 in Acute stroke Treatment) and the association of stroke subtypes with the SLE risk genotypes; *STAT4* and *HLA-DRB1*.

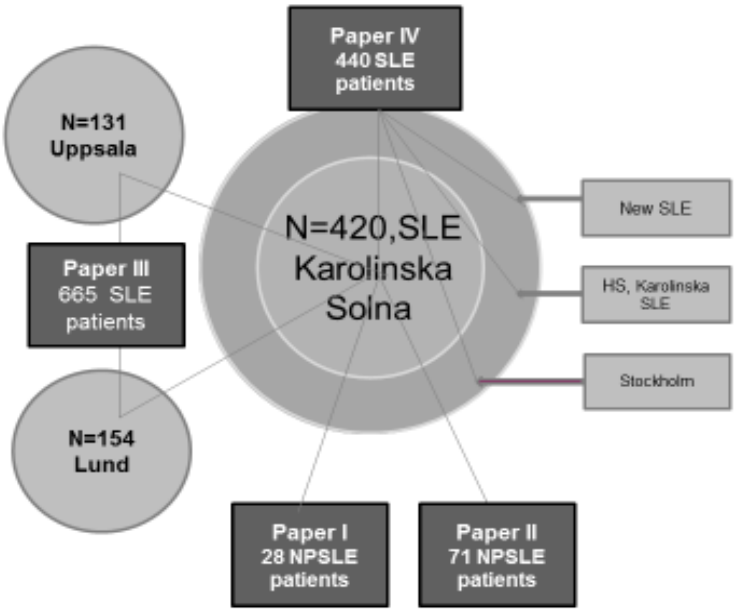
4 To determine the prevalence and classify seizures and epilepsy among patients with SLE and investigate the relationship between epilepsy and other manifestations of neuropsychiatric SLE.

4. MATERIALS AND METHODS

4.1. SUBJECTS AND STUDY DESIGN (I-IV)

The patients included in our studies are mainly from a cohort, which now comprised about 440 SLE patients who fulfilled criteria for SLE (10, 11) and attending the Rheumatology Clinic at the Karolinska University Hospital. (Figure 3). During time of our studies, the rheumatologist added new patients to this Hospital-based cohort from 1996 to 2012. Studies I and II were cross-sectional and the SLE-patients were referred to the neurology clinic for clinical investigation because of NPSLE symptoms. In this thesis we utilized blood and cerebrospinal fluid that were kept frozen at -70˚C in our in-house biobank. MRI and/or CT scans of the brain, EEG and other neurophysiological investigations were performed depending on the clinical presentation. All the patients in study IV were from this cohort. In study III the patients were included from the cohorts in Karolinska Solna (N=380), Uppsala (N=131) and from Lund (N=154). Studies III and IV had a retrospective design. The matched control samples used in studies I and II were from non-inflammatory neurological patients, mostly tension headache and in study I samples from relapsing remitting Multiple sclerosis (RRMS) patients were included.

Figure 3. Study design



All studies included were approved by the regional Ethics Committee and written informed consent was obtained from all study participants.

Paper I

Twenty-eight patients with neurological manifestations of SLE were included. APRIL and BAFF in paired plasma and CSF samples from NPSLE patients were quantified using a commercial ELISA kit, thirteen RRMS patients and seventeen OND patients acting as controls. Quantitative PCR was performed for APRIL and BAFF mRNA respectively for CSF and PB. The Fatigue Severity Scale (FSS) was used to measure the degree of fatigue.

Paper II

Plasma and/or CSF samples from 71 NPSLE patients and 58 control cases were analyzed. JCV DNA was analyzed using a quantitative PCR method. Paired CSF and plasma samples were obtained from 49 NPSLE patients and 44 of the control patients. At sampling ongoing medication were documented: corticosteroids (n=51), Hydroxychloroquine (n=22), Azathioprine (n=15), Methotrexate (n=3), Mycophenolate mofetil (n=3), Rituximab (n=3), Cyclosporine (n=1), Immunoglobulin (n=1), none for Cyclophosphamide, Chlorambucil or Reumacom. One of the patients had previously undergone autologous hematopoietic stem cell transplantation and plasmapheresis. Five patients had no treatment.

Paper III

Among 665 genotyped SLE patients from three large cohorts we identified 69 patients with stroke. The medical charts were reviewed and brain, cardiac and vascular imaging at the time of stroke was reinvestigated. The 56 ischemic stroke cases were possible to evaluate and categorize according to the TOAST subtypes. Evaluators were blinded to genotype. Association with STAT4 and HLA-DRB genotypes were explored. In the genetic comparisons the controls were either SLE patients free from previous ischemic cerebrovascular disease or general population controls.

Paper IV

The WHO epilepsy screening questionnaire was sent to all of 440 patients who were still alive or not lost during the follow-up. A totally of 368 questionnaires were dispatched, the patient who positive screened cases were offered neurological examination. The medical records of the patients were reviewed. Seizures were classified and NPSLE symptoms documented according to ACR nomenclature. Occurrence of autoantibodies and the antiphospholipid syndrome were recorded.

4.2. CLINICAL INSTRUMENTS – QUESTIONNAIRES

In paper I we used the fatigue severity scale, the most frequently used scale for SLE and MS patients for the assessment of subjective fatigue. It has high reliability, validity and internal consistency. It comprises a nine-item scale scored from one to seven; the mean value of the answered questions results in the final score (range 1-7) (18).
In paper IV we used the WHO screening questionnaire of epilepsy (WHO, Epilepsy management at a primary health level (WHO; 2000; Geneva; 2000) to discern possible signs or symptoms of epilepsy. This questionnaire includes thirteen statements to answer `yes´ or `no´; one positive answer is required for further investigation. Seizure disorders were classified according to the proposal by the International League against Epilepsy (ILAE) including the guidelines for Epidemiologic studies on Epilepsy (108-110). Acute symptomatic seizures were defined as suggested by the ILAE Task Force (120).

4.3. LABORATORY MEASUREMENTS

4.3.1. Cerebrospinal fluid and routine laboratory parameters

In papers I and II the CSF and blood work-up performed were as a part of routine analysis. Abnormal CSF parameters included a monocyte cell count > 5 cells/µL, IgG index >0.7 or signs of blood-brain barrier dysfunction. The assays were conducted at the Department of Clinical Chemistry, Karolinska University Hospital. Prepared and paired CSF and plasma samples were stored and kept frozen at -70^{0}C in our biobank at the Neurological Clinic until use.

4.3.2. Autoantibodies (I-IV*)*

Papers I and II. Autoantibody tests were performed as routine analyses at the Department of Clinical Immunology, Karolinska University Hospital. Antibodies to nuclear antigens (ANA) were analyzed on HEp-2 cells (Immunoconcepts, Sacramento, CA, USA). Antibodies to Sjögren's syndrome A/B (SSA/SSB), Sm, and RNP were analyzed using an ANA-profile enzyme-linked immunosorbent assay (ELISA; Pharmacia Diagnostics, Uppsala, Sweden), Innolia Immunoblot (Innogenetics, N.U. Ghent, Belgium), and Auto Immunodiffusion (Immuno Concepts).

For paper III anti-dsDNA was assayed by ELISA dsDNA (Pharmacia Diagnostics, Uppsala, Sweden). Autoantibodies against cardiolipin (IgG and IgM) and ß2glycoprotein I ($ß_2$GPI, IgG) were analyzed by ELISA (Orgentec, Mainz, Germany), in frozen serum samples at the time of inclusion into the genetic studies, i.e. after the stroke event.

For paper IV the antibodies to specific nuclear antigens (dsDNA, SSA/Ro52, SSA/Ro-60, SSB, Sm) and phospholipids cardiolipin (IgG, IgM) and $ß_2$glycoprotein I (IgG, IgM) were analyzed using a multiplexed bead technology (Luminex) using BioPlex 2200 system (Bio-Rad, Hercules, CA, USA) according to the specifications of the manufacturer. The cut-off for anti cardiolipin (aCL) and anti$ß_2$-glycoprotein 1 (a$ß_2$GP1) fulfill the 99th percentile as described (121). LA was determined using a modified Dilute Russel Viper Venom method (Biopool, Umea, Sweden) using Bioclot lupus anticoagulant.

The anti-phospholipid syndrome (APS) was defined by the presence of anti-phospholipid antibodies (a PL) and one or more thrombotic (venous, arterial or small vessel) episodes in any tissue or organ, or pregnancy-related morbidity. At least one clinical and one laboratory criterion (minimum of two positive tests taken at least 12 weeks apart) are required for APS classification (27).

4.3.3. Quantification of APRIL and BAFF by ELISA PCR

Levels of soluble APRIL and BAFF in paired plasma and CSF samples were quantified using a human APRIL ELISA kit (BenderMedSystem in Vienna, Austria) and a human BAFF ELISA kit (R&D Systems, Abingdon, UK). Quantitative PCR was performed for APRIL and BAFF mRNA respectively, for CSF and PB. APRIL primers were custom-designed by Applied Biosystems. BAFF primers and the endogenous control glyceraldehyde 3-phosphate

dehydrogenase (GAPDH) were purchased from Applied Biosystems. APRIL and BAFF mRNA levels were quantified and normalized for GAPDH expression (122, 123). For more details, see the section for subjects and methods in paper I.

4.3.4. JCV PCR analysis

For paper II total DNA was extracted from 200 ml of plasma or CSF using M48 Blood and Tissue Midi Kit (Qiagen, Hilden, Germany) in combination with the M48 Biorobot (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA obtained from the plasma samples was diluted in a volume equal to the starting volume, while DNA obtained from the CSF samples was adjusted to a final volume of 50 µl. Quantification of JCV DNA was performed by RealTime PCR (qPCR). The specific JCV primers and probe were designed to amplify a part of the VP 2 region (forward primer 50 CTGAACC AAAAGCTACATAGTAAGTAATGT-30, reverse primer 50CTAGTCCCCCAAAAGTGGAA-3, probe 50FAM-AGGTTCATGGGTGCCGC-30 MGB). The detection limit for JCV DNA in plasma was 200 copies/ml, and corresponding limit for CSF was 50 copies/ml. (124, 125). For further details, please refer to paper II.

4.3.5. Genotyping

In paper III, standard procedures were used to extract DNA from blood samples. Genotyping: The genotypes for SNP rs10181656 in intron 3 of the STAT4 gene were determined using the Goldengate assay (Illumina Inc., San Diego, CA, USA) (126). The genotype call rate was 97.0 % and the reproducibility 100% as determined by duplicate genotyping of 17 samples. An additional, partly overlapping, set of 156 subjects had previously been genotyped using the SNPstream system (Beckman-Coulter Inc., Fullerton, CA, USA). The concordance between the two methods was 100%. The genotypes of the SNP rs10181656 in controls conformed to Hardy-Weinberg equilibrium ($p > 0.05$). Patients were included in the genetic studies on average of 7.8 years after their first stroke episode (44, 127).

HLA-DRB1 genotyping was performed by sequence-specific primer PCR assay (SSP-PCR) (DR low-resolution kit; Olerup SSP, Saltsjöbaden, Sweden) and the PCR products were loaded into 2% agarose gels for electrophoresis. An interpretation table was

used to determine the specific genotype according to the manufacturer's instructions (126). The HLA-DRB1 allelic groups studied were DRB1*01, DRB1*03, DRB1*04, DRB1*07, DRB1*08, DRB1*09, DRB1*10, DRB1*11, DRB1*12, DRB1*13, DRB1*14, DRB1*15 and DRB1*16.

4.3.6. Statistical analyses

Statistical analyses were performed using non-parametric Wilcoxon signed ranks test, and correlations between demographic variables were analyzed with Spearman's rank test (Graphpad Prism 5.0, San Diego, CA). A probability value less than 0.05 was considered statistically significant in paper I. Standard descriptive statistics were used for presentation of the categorical variables and were summarized as counts and percentages. Continuous variables were expressed as median and interquartile range in papers I, II and IV. In paper IV statistical analyses were performed by calculating correlations between demographic variables and analyses between the groups were performed with Dunn's multiple comparison and Fisher's exact t-test using Graphpad Prism 5. A p-value <0.05 was considered statistically significant. In paper III clinical patient characteristics and allele frequencies were compared between groups with chi-squared tests or Fisher's exact test, depending on numbers of investigated individuals. Odds ratios (OR) and 95% confidence intervals (CI) were calculated from 2x2 contingency tables. Continuous variables were analysed using Mann-Whitney U-test or students T-test, depending on variable distribution. A p-value <0.05 was considered significant. Bonferroni corrections, based on 24 tests regarding STAT4 and 96 tests for HLA-DRB1 calculations, yielded $p<0.002$ for STAT4 and $p<0.0005$ for HLA-DRBI as corresponding significant p-values. Data processing was performed using JMP software (SAS Institute Inc. Carey, North Carolina).

5. RESULTS AND DISCUSSION

5.1. PAPER I

BAFF and APRIL are cytokines suggested to be of importance for autoimmune diseases. At the time our study was performed, a BAFF blocking antibody, Belimumab had shown efficacy in clinical trials in lupus patients. We aimed to investigate these B-cell survival molecules in the intrathecal and systemic compartments in SLE patients with neurological symptoms compared with controls.

NPSLE patients had higher levels of APRIL in CSF as compared to OND ($p<0.01$). No corresponding increase in APRIL mRNA levels was detected in CSF-MC. BAFF levels in plasma were higher in NPSLE than in OND ($p < 0.001$). BAFF mRNA expression in PBMC was also higher in NPSLE patients than in controls ($p < 0.05$). FSS score levels in NPSLE patients was with median 5.56 (range 4.56-6.67) and correlated significantly with APRIL levels in CSF ($p<0.05$).

Three patients displayed very high levels of APRIL in CSF and they all scored high on the FSS questionnaire (6.63–7.00), one of them having an acute exacerbation of NPSLE with cognitive symptoms and impaired vision at the time of sampling. Four patients displayed very high CSF BAFF levels, two of these being investigated during an acute exacerbation of NPSLE. Three of these four patients had pathological alterations in BBB function together with high levels of plasma BAFF. In two of the three patients with BBB dysfunction, a high level of BAFF mRNA expression in PBMC was noted.

Nearly half of the patients received immunosuppressive treatment such as rituximab, azathioprine, methotrexate, cyclophosphamide, and anti-malarias, at the time of sampling. A total of four had acute neuropsychiatric manifestations requiring hospitalization at the time of sampling. Headache was the most common symptom (57%). Cognitive symptoms were the second most common symptom (36%), followed by mood disorder (25%).

In conclusion, we found a correlation between higher scores in FSS with high APRIL levels in CSF for NPSLE patients. Higher BAFF levels in plasma and in peripheral blood for NPSLE patients compared to controls. Higher levels of APRIL in CSF for NPSLE were recorded compared to OND. These results indicate that intrathecal immunological activity contributes to fatigue in SLE. Our study has limitations, as it is a small study. We might also include patients with SLE without any neurological symptoms as controls. To get more power we should have had a more clear hypotheses from the beginning.

5.2. PAPER II

There are several clinical case reports of PML, a potentially fatal disease, in SLE patients, both in association with newer biologic immunosuppressive or immunomodulatory treatments, but also among patients treated with conventional disease-modifying agents. PML as a risk with newer biologicals became highlighted in 2004, when three of 3000 patients with MS/Crohn's disease treated with natalizumab developed PML due to reactivation of asymptomatic and latent JCV infection with subsequent development (128, 129).

Our intention for this study was to test the hypothesis that NPSLE patients who were treated with different types and duration of immunosuppressive medication would display reactivation of JCV and that detection of JCV in the systemic circulation or in CSF should in that case be possible.

CSF (n=69) and plasma (n=51) samples from 71 NPSLE patients and CSF (n=53) and plasma (n=50) from 58 controls were analyzed for presence of JCV DNA. Paired CSF and plasma samples were obtained from 49 of the NPSLE patients and 44 of the control patients. Sixty-three of the NPSLE patients had ongoing treatment with immunomodulatory medication at the time of sampling. Five NPSLE patients were treatment-naïve and eight of the patients had no immunomodulatory treatment at the time of sampling but had been treated with prior to sampling.

We did not find any CSF or plasma samples positive for JCV, in either patients or controls. All NPSLE patients were followed up clinically, after sampling, for a mean of 5.4 years (range 3-8) and none of them developed clinical signs of PML. Six SLE patients died during the follow-up time period; causes of death were cerebral vasculitis and pneumonia, CNS demyelination and pneumonia, cortical dementia, depression and cognitive impairment, ischemic heart disease and cerebrovascular disease.

In conclusion, our study could not confirm occurrence of JCV DNA in a cohort of SLE patients treated with several immunosuppressive medications from a Swedish hospital-based SLE cohort. However, considering the knowledge of possible reactivation of JCV for all the patients undergoing immunosuppressive therapies, clinicians should be observant for new clinical signs or symptoms from the CNS. The clinical, radiological evaluation, serology -and/or CSF JCV analyses should be performed as a standard.

5.3. PAPER III

In this study 56 patients were finally diagnosed with ischemic stroke, 13 cases of the original 69 were excluded due to history of hemorrhagic stroke, insufficient information for neuroimaging to fulfill the TOAST classification or insufficient genotype information after collection. These patients were diagnosed with SLE fulfilling at least four of the ACR criteria at a mean age of 34 (range 11-76) years and 91 % were female. The mean age at the time of the first stroke was 52 years (range 18-84). According to stroke subtypes distribution occurred 12% LAA, 21% CE, 16% SAO, 33% OE/APS and 16% UE. Patients with ischemic stroke of OE/APS or CE represented 31 of the 56 cases (55%). Patients with OE/APS-associated strokes were significantly younger than other stroke subtypes (median age 42 vs. 59 years) p=0.003. Strokes of OE/APS and CE origin, but not other stroke subtypes, were associated with the *STAT4* risk genotype. HLA-DRB1 alleles were not related to stroke subtype.

A high proportion (45%) of all patients with ischemic stroke in this study were positive for at least one of the investigated aPL. Before inclusion into the genetic studies venous thromboembolic disease occurred in 32%, IHD in 18% and ischemic peripheral vascular disease in 18% of patients with ischemic stroke. A further 28% suffered from heart valve affection and half of them had valve prosthesis.

In conclusion, our study confirms several previous studies, which have demonstrated that aPL/APS plays an important role for ischemic heart and cerebrovascular disease in SLE. The incidence of ischemic stroke is two-to three-fold among enhanced among SLE patients as compared to the general population and the relative risk is more increased among younger SLE patients < 50 years. In our study the APS/OE sub-group had median age of 42 years, and patients with heart valve disease were common. Another interesting finding was the association of STAT4 genotype to ischemic stroke in both APS/OE and CE subtypes.

There are limitations with our study as it has a retrospective design and a relatively small size with only Swedish Caucasians participants. Some patients were excluded due to lack of genetic data and due to insufficient information to make the classification according to TOAST subclasses. Nevertheless, based on our findings we suggest that all SLE patients should be thoroughly investigated at diagnosis for aPL and further investigations with echocardiography should be performed to detect cardiac/heart valve risk factors for ischemic stroke.

5.4. PAPER IV

Epilepsy and/or seizures have been reported to occur in variable proportions of SLE patients ranging from 3%-64%, due to differences in study population and methodology. Our study based in a hospital SLE cohort included a totally of 440 patients. At the start of the study 14% of patients in the cohort had died and 2.7% were lost to follow-up of other reasons, resulting in the questionnaire being sent to 368 patients, of which 312 (85%) responded. Of these, 42% screened positive. Thirty-six SLE patients (11.5%) fulfilled the ILAE criteria for epilepsy, which is more common than in the general Swedish population (0.7%). Mean age at seizure onset was 32 years (range 1-84) and the mean age at SLE diagnosis was 36 years (range 11-67), four of these were males (11%).

The finding that seizures were of focal onset in 83% is notable, differing from previous studies of SLE population. Almost all of these patients 35/36 screened positively in the WHO questionnaire, one patient developed epilepsy during our data collection. Eight of these 36 patients (22%) overlapped with SLE diagnosis. Sixteen (44%) had onset before SLE diagnosis and 12 patients had their first seizure after SLE diagnosis. Manifestations of NPSLE occurred in 50%. Cerebrovascular disease was more than twice as common ($p=0.001$) and psychosis increased three-fold ($p=0.0006$) in NPSLE patients with epilepsy versus NPSLE patients without epilepsy. APS was more common in patients with epilepsy compared to epilepsy-free SLE patients with or without NPSLE ($p=0.02$). In 50% of patients with epilepsy no other etiology than SLE, was detected.

In conclusion we report an 11.5% prevalence of epilepsy in a large and well-characterized hospital-based SLE cohort, the majority of our patients being classified with focal epilepsy. APS was more common in patients with epilepsy compared to epilepsy-free SLE patients with or without NPSLE ($p=0.02$). Cerebrovascular disease was highly significantly more common. In 50% of patients with epilepsy no other etiology than SLE, was detected.

The power of our study from the start was the direct communication with our patients regarding the questionnaire. We could identify all possible cases with seizures and proceed to a structured interview and clinical investigation. We noticed the difference in establishing an epilepsy diagnosis. According the 11 ACE classification criteria for SLE, the patients also fulfilled one of the basic criteria due to the seizure. Several of these patients with one unprovoked seizure continued with further epileptic activity and mostly with focal seizures

that qualified for epilepsy diagnosis. Our study provides additional support that immunological mechanisms are important for the pathogenesis of epilepsy.

A limitation of this study is that it is retrospective design and that SLE patients were identified in hospital-based registries that might lead to generalized conclusions for SLE and epilepsy. However, the Rheumatology Departments in our study are referral centers for SLE patients, a population base of approximately 1.2 million inhabitants, thus representing a large population.

6. GENERAL DISCUSSION

The studies in this thesis are based on the carefully diagnosed SLE patients attending the in Rheumatology Clinic at Karolinska University Hospital with suspected actual or earlier neurological manifestations and/or psychiatric and/or cognitive symptoms or impairments. Neuropsychiatric symptoms in SLE are diverse, nineteen case definition being published in 1999 (11), as earlier discussed in these thesis, including both central and peripheral involvement. This is probably not the complete catalogue, for example posterior reversible encephalopathy syndrome (PRES) is reported to med more common in SLE patients. Two NPSLE manifestations are included in the ACR criteria for SLE diagnosis, namely epilepsy or psychosis. It is obvious that involvement of the nervous system is an important feature of SLE, and it is thereby also important to better diagnose and understand how this systemic disease affects the nervous system.

The search for informative biomarkers relating to diagnosis, disease activity or treatment responses have been intense for a number of years. A large number of markers relating to different pathways have been suggested, but very few have been replicated and validated.

There are limitations in our studies, namely a small size for power and due to heterogeneous neurological manifestations difficulties in making comparisons i.e. with acute and chronic neurological clinical cases. We should also have included SLE patients without neurological manifestations as controls. Every patient was indeed carefully diagnosed, investigated both by a rheumatologist and a neurologist. The preferred outcome of our studies should, however, be to provide better information for the clinician, to assist them in making a better diagnostic work-up and better informed knowledge about patient treatment options.

Our second study aimed to assess the presence and viral load of JC polyomavirus DNA in cerebrospinal fluid and plasma from NPSLE patients compared with controls. Our negative findings considering JCV DNA in CSF or in peripheral blood in samples for all these patients treated with several immune suppressive medications and controls were encouraging. Seventy-one SLE patients with well-documented immunosuppressive medications were included in this study. One may ask if the methods we used to detect the levels of JCV DNA were sensitive enough to answer the question of probable risk for the future reactivation of JCV, leading to PML. All of these laboratory methods are continuously improved and actual levels for measurement should be followed at the time of each individual investigation for suspected JC virus involvement in CNS. The previous systematic literature

review by Henegar *et al.* (130) refers to larger population-based observational studies regarding the risk of PML in SLE patients versus non-SLE patients, treated with similar immunosuppressive medicals used for SLE management. These indicate that PML is a very rare disease in SLE with the incidence range (1.0-2.4) per 100 000 person years and suggests that there appears to be an increased risk of PML associated with SLE in the general population. Due to the knowledge of PML, often with lethal outcome, this risk should be studied further when treating our patients with any of these immunosuppressive pharmaceuticals.

The third study included a total of 665 patients from three leading centers for SLE research and we could determine ischemic stroke in 56 of 69 suspected cases. Information for classification of subtypes of stroke according to TOAST was based on available medical records and the evaluators for were blinded for genotypes of the patients. In some cases lack of information and difficulties in obtaining original brain images to confirm the findings in some suggested cases caused exclusion of patients, leading to smaller size and lower power for our study.

The results of our previous studies on association with STAT4 and HLA-DRB1 risk genotypes to both aPL and to vascular disease indicated an enhanced risk of vascular events (44). We aimed to investigate these risk alleles among ischemic stroke subtypes compared with controls. Our study demonstrates that more than half of the ischemic strokes were associated with OE/APS or CE origin. Patients with APS-related stroke were younger. Compared to the prospective study by Mikdashi *et al.* (131), they had a different view of the relations between APS and anti-cardiolipin. The lack of formal APS diagnosis in one study, ethnicity and culture may explain the discrepancy. Our study has limitations. It is retrospective in design and all participants were European Caucasians, which may obstruct the general applicability.

In the fourth study, we determined the prevalence of epilepsy in our SLE cohort. We report prevalence of epilepsy of 11.5%, a much higher level in SLE as compared to the population at large. Our methodology, using the screening questionnaire, enabled us to collect a large number of patients with suspected seizures, with a high number completing the questionnaire. Combining individual structured interviews and medical chart reviews we could confirm or discard a diagnosis of epilepsy according to ILEA criteria (108-110), exclude acute symptomatic seizures (109) and also define a possible presence of other NPSLE manifestations (11). Concomitant conditions unrelated to SLE were investigated. Autoantibody levels and LA were determined. The majority of the patients with epilepsy had

focal seizures (83%), in contrast to a prior study by Appenzeller *et al*. (73) who reported generalized seizures for the great majority (88%) as well as González-Duarte *et al.* who reported tonic-clonic seizures in 77%. The large study by Hanly *et al.* (132, 133) reported 60% as primary generalized and 31% as focal. These three studies were all prospective with another kind of study design, with various times of follow-up. Our study has a retrospective design and the patients were identified in hospital-based registries which might explain the differences. However, these patients were well-characterized, many patients being followed-up for many years at the Rheumatology Departments. Information from medical records was available together with patients fulfilling the questionnaire and clinical evidence of recurred unprovoked seizures. We could determine the type of epilepsy and count the prevalence in this cohort. Our data suggest a multifactorial background to epilepsy with association with cerebrovascular disease, psychosis and APS.

6.1. FUTURE DIRECTIONS

One of the main focuses in this thesis was to provide deeper understanding of NPSLE symptoms and draw to conclusions that might improve the clinical care of this patients, as well as indicating ideas for both short- and long-term evaluation of these patients.
The following options could benefit this purpose.

- A standardized protocol should be created and used for prospective follow-up both for clinical use and for possible future studies, including MRI investigation for NPSLE patients, and a fatigue scale – for each patients repetitively and for to use interactively in the waiting room. In addition, an EP screening questionnaire for patients with a history of suspected seizures
- CSF investigations – for patients with a new NP manifestation, or in relapses of CNS symptoms including the latest items for detecting of more specific changes in the intrathecal compartment.
- SLE patients with positive aPL and/or those with APS should be observed attentively for CVL risks
- Investigate SLE patients early with echocardiography to detect possible cardiological risk factors for neurological complications
- Include the investigation of JCV serology as a routine analyses for SLE patients treated with immunosuppressive medicine, as a baseline

- To provide more resources for neuropsychological assessments and counselling
- Include the patients with NPSLE syndromes not only in national SLE register in Sweden, but also as an subgroup in the Neuro register in Sweden to benefit further research of autoimmune diseases that influence CNS or/and co-operate at a national level to get a better evidence and power for studies and conclusions.
- Continue with further studies in NPSLE and select one syndrome at a time as focus for each study instead of lumping all NPSLE syndromes together.

7. CONCLUSIONS

- APRIL protein levels in CSF from NPSLE patients were significantly higher compared to levels in CSF from OND patients
- Fatigue correlated to higher APRIL levels in CSF of NPSLE patients
- All CSF and plasma samples from both NPSLE patients and controls investigated were negative for JCV DNA
- Occurrence of JCV DNA was not common in the blood and CSF among patients with NPSLE patients receiving immunomodulatory treatments
- Swedish SLE patients with ischemic stroke mainly had an etiology classified as due to other determined category (OE) such as presence of APS or due to cardio-embolic origin (CE) according to the TOAST classification.
- These two subtypes of stroke were associated to the STAT4 genotype.
- Patients with APS-associated stroke were younger than non-APS stroke patients.
- The validated epilepsy prevalence was 11.5% in a large and well-characterized hospital-based SLE cohort and a majority of the cases could be classified as focal epilepsy. Cerebrovascular disease and APS were associated with epilepsy.

The overall evaluation and the result of our clinical studies is that SLE is a multifactorial and serious systemic disease that involves both the central and peripheral nervous system with heterogeneous manifestations. Immunological, vascular, hematological, and cardiovascular and brain lesions due several etiologies contribute to the occurrence of different neurological symptoms. Based on our findings we suggest that all SLE patients with neurological manifestations such as CVL and epilepsy should be thoroughly investigated at diagnosis for aPL, echocardiographic investigation should be performed to detect risk factors for ischemic stroke, and brain imaging and CSF analysis should be a standard.

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9. REFERENCES

1. Tsokos GC. Systemic lupus erythematosus. The New England journal of medicine. 2011;365(22):2110-21.

2. Hanly JG. Diagnosis and management of neuropsychiatric SLE. Nature reviews Rheumatology. 2014;10(6):338-47.

3. Govoni M, Bortoluzzi A, Padovan M, Silvagni E, Borrelli M, Donelli F, et al. The diagnosis and clinical management of the neuropsychiatric manifestations of lupus. Journal of autoimmunity. 2016.

4. Lisnevskaia L, Murphy G, Isenberg D. Systemic lupus erythematosus. Lancet. 2014;384(9957):1878-88.

5. Pons-Estel GJ, Alarcon GS, Scofield L, Reinlib L, Cooper GS. Understanding the epidemiology and progression of systemic lupus erythematosus. Seminars in arthritis and rheumatism. 2010;39(4):257-68.

6. Stahl-Hallengren C, Jonsen A, Nived O, Sturfelt G. Incidence studies of systemic lupus erythematosus in Southern Sweden: increasing age, decreasing frequency of renal manifestations and good prognosis. The Journal of rheumatology. 2000;27(3):685-91.

7. Simard JF, Sjowall C, Ronnblom L, Jonsen A, Svenungsson E. Systemic lupus erythematosus prevalence in Sweden in 2010: what do national registers say? Arthritis care & research. 2014;66(11):1710-7.

8. Rahman A, Isenberg DA. Systemic lupus erythematosus. The New England journal of medicine. 2008;358(9):929-39.

9. Somers EC, Marder W, Cagnoli P, Lewis EE, DeGuire P, Gordon C, et al. Population-based incidence and prevalence of systemic lupus erythematosus: the Michigan Lupus Epidemiology and Surveillance program. Arthritis & rheumatology. 2014;66(2):369-78.

10. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis and rheumatism. 1982;25(11):1271-7.

11. The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. Arthritis and rheumatism. 1999;42(4):599-608.

12. Brey RL, Holliday SL, Saklad AR, Navarrete MG, Hermosillo-Romo D, Stallworth CL, et al. Neuropsychiatric syndromes in lupus: prevalence using standardized definitions. Neurology. 2002;58(8):1214-20.

13. Petri M, Orbai AM, Alarcon GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis and rheumatism. 2012;64(8):2677-86.

14. Ippolito A, Petri M. An update on mortality in systemic lupus erythematosus. Clinical and experimental rheumatology. 2008;26(5 Suppl 51):S72-9.

15. Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. Medicine. 2003;82(5):299-308.

16. Bernatsky S, Clarke A, Gladman DD, Urowitz M, Fortin PR, Barr SG, et al. Mortality related to cerebrovascular disease in systemic lupus erythematosus. Lupus. 2006;15(12):835-9.

17. Barr SG, Zonana-Nacach A, Magder LS, Petri M. Patterns of disease activity in systemic lupus erythematosus. Arthritis and rheumatism. 1999;42(12):2682-8.

18. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Archives of neurology. 1989;46(10):1121-3.

19. Bruce IN, Mak VC, Hallett DC, Gladman DD, Urowitz MB. Factors associated with fatigue in patients with systemic lupus erythematosus. Annals of the rheumatic diseases. 1999;58(6):379-81.

20. Sherer Y, Gorstein A, Fritzler MJ, Shoenfeld Y. Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients. Seminars in arthritis and rheumatism. 2004;34(2):501-37.

21. To CH, Petri M. Is antibody clustering predictive of clinical subsets and damage in systemic lupus erythematosus? Arthritis and rheumatism. 2005;52(12):4003-10.

22. Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of systemic lupus erythematosus in northern Sweden. Arthritis research & therapy. 2011;13(1):R30.

23. Scolding NJ, Joseph FG. The neuropathology and pathogenesis of systemic lupus erythematosus. Neuropathology and applied neurobiology. 2002;28(3):173-89.

24. Andreoli L, Chighizola CB, Banzato A, Pons-Estel GJ, Ramire de Jesus G, Erkan D. Estimated frequency of antiphospholipid antibodies in patients with pregnancy morbidity, stroke, myocardial infarction, and deep vein thrombosis: a critical review of the literature. Arthritis care & research. 2013;65(11):1869-73.

25. Hughes GR. Thrombosis, abortion, cerebral disease, and the lupus anticoagulant. British medical journal. 1983;287(6399):1088-9.

26. Hughes GR. The antiphospholipid syndrome: ten years on. Lancet. 1993;342(8867):341-4.

27. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). Journal of thrombosis and haemostasis : JTH. 2006;4(2):295-306.

28. Lood C, Gullstrand B, Truedsson L, Olin AI, Alm GV, Ronnblom L, et al. C1q inhibits immune complex-induced interferon-alpha production in plasmacytoid dendritic cells: a novel link between C1q deficiency and systemic lupus erythematosus pathogenesis. Arthritis and rheumatism. 2009;60(10):3081-90.

29. Truedsson L, Bengtsson AA, Sturfelt G. Complement deficiencies and systemic lupus erythematosus. Autoimmunity. 2007;40(8):560-6.

30. Lood C, Amisten S, Gullstrand B, Jonsen A, Allhorn M, Truedsson L, et al. Platelet transcriptional profile and protein expression in patients with systemic lupus erythematosus: up-regulation of the type I interferon system is strongly associated with vascular disease. Blood. 2010;116(11):1951-7.

31. Santer DM, Yoshio T, Minota S, Moller T, Elkon KB. Potent induction of IFN-alpha and chemokines by autoantibodies in the cerebrospinal fluid of patients with neuropsychiatric lupus. Journal of immunology (Baltimore, Md : 1950). 2009;182(2):1192-201.

32. Ronnblom L, Eloranta ML, Alm GV. The type I interferon system in systemic lupus erythematosus. Arthritis and rheumatism. 2006;54(2):408-20.

33. Svenungsson E, Gunnarsson I, Fei GZ, Lundberg IE, Klareskog L, Frostegard J. Elevated triglycerides and low levels of high-density lipoprotein as markers of disease activity in association with up-regulation of the tumor necrosis factor alpha/tumor necrosis factor receptor system in systemic lupus erythematosus. Arthritis and rheumatism. 2003;48(9):2533-40.

34. Leffler J, Bengtsson AA, Blom AM. The complement system in systemic lupus erythematosus: an update. Annals of the rheumatic diseases. 2014;73(9):1601-6.

35. Tangye SG, Bryant VL, Cuss AK, Good KL. BAFF, APRIL and human B cell disorders. Seminars in immunology. 2006;18(5):305-17.

36. Koyama T, Tsukamoto H, Miyagi Y, Himeji D, Otsuka J, Miyagawa H, et al. Raised serum APRIL levels in patients with systemic lupus erythematosus. Annals of the rheumatic diseases. 2005;64(7):1065-7.

37. Cheema GS, Roschke V, Hilbert DM, Stohl W. Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases. Arthritis and rheumatism. 2001;44(6):1313-9.

38. Jonsson MV, Szodoray P, Jellestad S, Jonsson R, Skarstein K. Association between circulating levels of the novel TNF family members APRIL and BAFF and lymphoid organization in primary Sjogren's syndrome. Journal of clinical immunology. 2005;25(3):189-201.

39. Stohl W. Systemic lupus erythematosus and its ABCs (APRIL/BLyS complexes). Arthritis research & therapy. 2010;12(2):111.

40. Mackay F, Schneider P, Rennert P, Browning J. BAFF AND APRIL: a tutorial on B cell survival. Annual review of immunology. 2003;21:231-64.

41. Bossen C, Schneider P. BAFF, APRIL and their receptors: structure, function and signaling. Seminars in immunology. 2006;18(5):263-75.

42. Espinosa G, Cervera R. Belimumab, a BLyS-specific inhibitor for the treatment of systemic lupus erythematosus. Drugs of today (Barcelona, Spain : 1998). 2010;46(12):891-9.

43. Belimumab: anti-BLyS monoclonal antibody; Benlysta; BmAb; LymphoStat-B. Drugs in R&D. 2010;10(1):55-65.

44. Svenungsson E, Gustafsson J, Leonard D, Sandling J, Gunnarsson I, Nordmark G, et al. A STAT4 risk allele is associated with ischaemic cerebrovascular events and anti-phospholipid antibodies in systemic lupus erythematosus. Annals of the rheumatic diseases. 2010;69(5):834-40.

45. Kaplan MH. STAT4: a critical regulator of inflammation in vivo. Immunologic research. 2005;31(3):231-42.

46. Crow MK. Collaboration, genetic associations, and lupus erythematosus. The New England journal of medicine. 2008;358(9):956-61.

47. Bryan AR, Wu EY. Complement deficiencies in systemic lupus erythematosus. Current allergy and asthma reports. 2014;14(7):448.

48. Joseph FG, Lammie GA, Scolding NJ. CNS lupus: a study of 41 patients. Neurology. 2007;69(7):644-54.

49. Cha JH, Furie K, Kay J, Walensky RP, Mullins ME, Hedley-Whyte ET. Case records of the Massachusetts General Hospital. Case 39-2006. A 24-year-old woman with systemic lupus erythematosus, seizures, and right arm weakness. The New England journal of medicine. 2006;355(25):2678-89.

50. Toledano P, Sarbu N, Espinosa G, Bargallo N, Cervera R. Neuropsychiatric systemic lupus erythematosus: magnetic resonance imaging findings and correlation with clinical and immunological features. Autoimmunity reviews. 2013;12(12):1166-70.

51. Ho RC, Thiaghu C, Ong H, Lu Y, Ho CS, Tam WW, et al. A meta-analysis of serum and cerebrospinal fluid autoantibodies in neuropsychiatric systemic lupus erythematosus. Autoimmunity reviews. 2016;15(2):124-38.

52. Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. Epilepsia. 2005;46(11):1724-43.

53. Jonsen A, Bengtsson AA, Nived O, Ryberg B, Truedsson L, Ronnblom L, et al. The heterogeneity of neuropsychiatric systemic lupus erythematosus is reflected in lack of association with cerebrospinal fluid cytokine profiles. Lupus. 2003;12(11):846-50.

54. Omdal R, Mellgren SI, Koldingsnes W, Jacobsen EA, Husby G. Fatigue in patients with systemic lupus erythematosus: lack of associations to serum cytokines, antiphospholipid antibodies, or other disease characteristics. The Journal of rheumatology. 2002;29(3):482-6.

55. Jeltsch-David H, Muller S. Neuropsychiatric systemic lupus erythematosus: pathogenesis and biomarkers. Nature reviews Neurology. 2014;10(10):579-96.

56. Appenzeller S, Rondina JM, Li LM, Costallat LT, Cendes F. Cerebral and corpus callosum atrophy in systemic lupus erythematosus. Arthritis and rheumatism. 2005;52(9):2783-9.

57. Jung RE, Segall JM, Grazioplene RG, Qualls C, Sibbitt WL, Roldan CA. Cortical thickness and subcortical gray matter reductions in neuropsychiatric systemic lupus erythematosus. PloS one. 2010;5(3):e9302.

58. Roldan CA, Shively BK, Crawford MH. An echocardiographic study of valvular heart disease associated with systemic lupus erythematosus. The New England journal of medicine. 1996;335(19):1424-30.

59. Roldan CA, Gelgand EA, Qualls CR, Sibbitt WL, Jr. Valvular heart disease as a cause of cerebrovascular disease in patients with systemic lupus erythematosus. The American journal of cardiology. 2005;95(12):1441-7.

60. Roldan CA, Qualls CR, Sopko KS, Sibbitt WL, Jr. Transthoracic versus transesophageal echocardiography for detection of Libman-Sacks endocarditis: a randomized controlled study. The Journal of rheumatology. 2008;35(2):224-9.

61. Zuily S, Regnault V, Selton-Suty C, Eschwege V, Bruntz JF, Bode-Dotto E, et al. Increased risk for heart valve disease associated with antiphospholipid antibodies in patients with systemic lupus erythematosus: meta-analysis of echocardiographic studies. Circulation. 2011;124(2):215-24.

62. Colasanti T, Delunardo F, Margutti P, Vacirca D, Piro E, Siracusano A, et al. Autoantibodies involved in neuropsychiatric manifestations associated with systemic lupus erythematosus. Journal of neuroimmunology. 2009;212(1-2):3-9.

63. Williams RC, Jr., Sugiura K, Tan EM. Antibodies to microtubule-associated protein 2 in patients with neuropsychiatric systemic lupus erythematosus. Arthritis and rheumatism. 2004;50(4):1239-47.

64. Zandman-Goddard G, Chapman J, Shoenfeld Y. Autoantibodies involved in neuropsychiatric SLE and antiphospholipid syndrome. Seminars in arthritis and rheumatism. 2007;36(5):297-315.

65. Trysberg E, Nylen K, Rosengren LE, Tarkowski A. Neuronal and astrocytic damage in systemic lupus erythematosus patients with central nervous system involvement. Arthritis and rheumatism. 2003;48(10):2881-7.

66. Sciascia S, Bertolaccini ML, Roccatello D, Khamashta MA, Sanna G. Autoantibodies involved in neuropsychiatric manifestations associated with systemic lupus erythematosus: a systematic review. Journal of neurology. 2014;261(9):1706-14.

67. Arinuma Y, Yanagida T, Hirohata S. Association of cerebrospinal fluid anti-NR2 glutamate receptor antibodies with diffuse neuropsychiatric systemic lupus erythematosus. Arthritis and rheumatism. 2008;58(4):1130-5.

68. DeGiorgio LA, Konstantinov KN, Lee SC, Hardin JA, Volpe BT, Diamond B. A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. Nature medicine. 2001;7(11):1189-93.

69. von Engelhardt J, Doganci B, Seeburg PH, Monyer H. Synaptic NR2A- but not NR2B-Containing NMDA Receptors Increase with Blockade of Ionotropic Glutamate Receptors. Frontiers in molecular neuroscience. 2009;2:19.

70. Lauvsnes MB, Omdal R. Systemic lupus erythematosus, the brain, and anti-NR2 antibodies. Journal of neurology. 2012;259(4):622-9.

71. Vikerfors A, Johansson AB, Gustafsson JT, Jonsen A, Leonard D, Zickert A, et al. Clinical manifestations and anti-phospholipid antibodies in 712 patients with systemic lupus erythematosus: evaluation of two diagnostic assays. Rheumatology (Oxford, England). 2013;52(3):501-9.

72. Liimatainen S, Peltola M, Fallah M, Kharazmi E, Haapala AM, Peltola J. The high prevalence of antiphospholipid antibodies in refractory focal epilepsy is related to recurrent seizures. European journal of neurology : the official journal of the European Federation of Neurological Societies. 2009;16(1):134-41.

73. Appenzeller S, Cendes F, Costallat LT. Epileptic seizures in systemic lupus erythematosus. Neurology. 2004;63(10):1808-12.

74. George-Chandy A, Trysberg E, Eriksson K. Raised intrathecal levels of APRIL and BAFF in patients with systemic lupus erythematosus: relationship to neuropsychiatric symptoms. Arthritis research & therapy. 2008;10(4):R97.

75. Morrison E, Carpentier S, Shaw E, Doucette S, Hanly JG. Neuropsychiatric systemic lupus erythematosus: association with global disease activity. Lupus. 2014;23(4):370-7.

76. Fragoso-Loyo H, Cabiedes J, Orozco-Narvaez A, Davila-Maldonado L, Atisha-Fregoso Y, Diamond B, et al. Serum and cerebrospinal fluid autoantibodies in patients with neuropsychiatric lupus erythematosus. Implications for diagnosis and pathogenesis. PloS one. 2008;3(10):e3347.

77. West SG, Emlen W, Wener MH, Kotzin BL. Neuropsychiatric lupus erythematosus: a 10-year prospective study on the value of diagnostic tests. The American journal of medicine. 1995;99(2):153-63.

78. Joseph FG, Scolding NJ. Neurolupus. Practical neurology. 2010;10(1):4-15.

79. Graham JW, Jan W. MRI and the brain in systemic lupus erythematosus. Lupus. 2003;12(12):891-6.

80. Kovacs JA, Urowitz MB, Gladman DD, Zeman R. The use of single photon emission computerized tomography in neuropsychiatric SLE: a pilot study. The Journal of rheumatology. 1995;22(7):1247-53.

81. Hanly JG, Urowitz MB, Su L, Bae SC, Gordon C, Wallace DJ, et al. Prospective analysis of neuropsychiatric events in an international disease inception cohort of patients with systemic lupus erythematosus. Annals of the rheumatic diseases. 2010;69(3):529-35.

82. Sanna G, Bertolaccini ML, Cuadrado MJ, Laing H, Khamashta MA, Mathieu A, et al. Neuropsychiatric manifestations in systemic lupus erythematosus: prevalence and association with antiphospholipid antibodies. The Journal of rheumatology. 2003;30(5):985-92.

83. Cuadrado MJ, Sanna G. Headache and systemic lupus erythematosus. Lupus. 2003;12(12):943-6.

84. Ainiala H, Loukkola J, Peltola J, Korpela M, Hietaharju A. The prevalence of neuropsychiatric syndromes in systemic lupus erythematosus. Neurology. 2001;57(3):496-500.

85. Cimaz R, Meroni PL, Shoenfeld Y. Epilepsy as part of systemic lupus erythematosus and systemic antiphospholipid syndrome (Hughes syndrome). Lupus. 2006;15(4):191-7.

86. Ullberg T, Zia E, Petersson J, Norrving B. Changes in functional outcome over the first year after stroke: an observational study from the Swedish stroke register. Stroke; a journal of cerebral circulation. 2015;46(2):389-94.

87. Feigin VL, Lawes CM, Bennett DA, Anderson CS. Stroke epidemiology: a review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. Lancet neurology. 2003;2(1):43-53.

88. Vaartjes I, O'Flaherty M, Capewell S, Kappelle J, Bots M. Remarkable decline in ischemic stroke mortality is not matched by changes in incidence. Stroke; a journal of cerebral circulation. 2013;44(3):591-7.

89. Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, et al. Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010. Lancet. 2014;383(9913):245-54.

90. Heuschmann PU, Di Carlo A, Bejot Y, Rastenyte D, Ryglewicz D, Sarti C, et al. Incidence of stroke in Europe at the beginning of the 21st century. Stroke; a journal of cerebral circulation. 2009;40(5):1557-63.

91. Hallstrom B, Jonsson AC, Nerbrand C, Norrving B, Lindgren A. Stroke incidence and survival in the beginning of the 21st century in southern Sweden: comparisons with the late 20th century and projections into the future. Stroke; a journal of cerebral circulation. 2008;39(1):10-5.

92. Holmqvist M, Simard JF, Asplund K, Arkema EV. Stroke in systemic lupus erythematosus: a meta-analysis of population-based cohort studies. RMD open. 2015;1(1):e000168.

93. Wiseman SJ, Ralston SH, Wardlaw JM. Cerebrovascular Disease in Rheumatic Diseases: A Systematic Review and Meta-Analysis. Stroke; a journal of cerebral circulation. 2016;47(4):943-50.

94. Mok CC, Ho LY, To CH. Annual incidence and standardized incidence ratio of cerebrovascular accidents in patients with systemic lupus erythematosus. Scandinavian journal of rheumatology. 2009;38(5):362-8.

95. Timlin H, Petri M. Transient ischemic attack and stroke in systemic lupus erythematosus. Lupus. 2013;22(12):1251-8.

96. Adams HP, Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke; a journal of cerebral circulation. 1993;24(1):35-41.

97. Kolominsky-Rabas PL, Weber M, Gefeller O, Neundoerfer B, Heuschmann PU. Epidemiology of ischemic stroke subtypes according to TOAST criteria: incidence, recurrence, and long-term survival in ischemic stroke subtypes: a population-based study. Stroke; a journal of cerebral circulation. 2001;32(12):2735-40.

98. Petty GW, Brown RD, Jr., Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO. Ischemic stroke subtypes : a population-based study of functional outcome, survival, and recurrence. Stroke; a journal of cerebral circulation. 2000;31(5):1062-8.

99. Cimaz R, Guerrini R. Epilepsy in lupus. Lupus. 2008;17(9):777-9.

100. Mikdashi J, Krumholz A, Handwerger B. Factors at diagnosis predict subsequent occurrence of seizures in systemic lupus erythematosus. Neurology. 2005;64(12):2102-7.

101. Mayes B, Brey RL. Evaluation and treatment of seizures in patients with systemic lupus erythematosus. Journal of clinical rheumatology : practical reports on rheumatic & musculoskeletal diseases. 1996;2(6):336-45.

102. Toubi E, Khamashta MA, Panarra A, Hughes GR. Association of antiphospholipid antibodies with central nervous system disease in systemic lupus erythematosus. The American journal of medicine. 1995;99(4):397-401.

103. Hanly JG, McCurdy G, Fougere L, Douglas JA, Thompson K. Neuropsychiatric events in systemic lupus erythematosus: attribution and clinical significance. The Journal of rheumatology. 2004;31(11):2156-62.

104. Sibbitt WL, Jr., Brandt JR, Johnson CR, Maldonado ME, Patel SR, Ford CC, et al. The incidence and prevalence of neuropsychiatric syndromes in pediatric onset systemic lupus erythematosus. The Journal of rheumatology. 2002;29(7):1536-42.

105. Hanly JG, Urowitz MB, Su L, Gordon C, Bae SC, Sanchez-Guerrero J, et al. Seizure disorders in systemic lupus erythematosus results from an international, prospective, inception cohort study. Annals of the rheumatic diseases. 2012;71(9):1502-9.

106. Andrade RM, Alarcon GS, Gonzalez LA, Fernandez M, Apte M, Vila LM, et al. Seizures in patients with systemic lupus erythematosus: data from LUMINA, a multiethnic cohort (LUMINA LIV). Annals of the rheumatic diseases. 2008;67(6):829-34.

107. Adelow C, Andersson T, Ahlbom A, Tomson T. Unprovoked seizures in multiple sclerosis and systemic lupus erythematosus: a population-based case-control study. Epilepsy research. 2012;101(3):284-7.

108. Guidelines for epidemiologic studies on epilepsy. Commission on Epidemiology and Prognosis, International League Against Epilepsy. Epilepsia. 1993;34(4):592-6.

109. Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia. 2005;46(4):470-2.

110. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014;55(4):475-82.

111. Sinclair DB, Snyder TJ. Corticosteroids for the treatment of Landau-kleffner syndrome and continuous spike-wave discharge during sleep. Pediatric neurology. 2005;32(5):300-6.

112. Berger JR. Progressive multifocal leukoencephalopathy and newer biological agents. Drug safety. 2010;33(11):969-83.

113. Aksamit AJ, Jr. Progressive multifocal leukoencephalopathy. Continuum (Minneapolis, Minn). 2012;18(6 Infectious Disease):1374-91.

114. De Gascun CF, Carr MJ. Human polyomavirus reactivation: disease pathogenesis and treatment approaches. Clinical & developmental immunology. 2013;2013:373579.

115. Molloy ES, Calabrese LH. Progressive multifocal leukoencephalopathy: a national estimate of frequency in systemic lupus erythematosus and other rheumatic diseases. Arthritis and rheumatism. 2009;60(12):3761-5.

116. Molloy ES, Calabrese LH. Progressive multifocal leukoencephalopathy associated with immunosuppressive therapy in rheumatic diseases: evolving role of biologic therapies. Arthritis and rheumatism. 2012;64(9):3043-51.

117. Govindappa V, Hicks S, Wichter M, Jolly M. Progressive multifocal leukoencephalopathy in systemic lupus erythematosus. Arthritis and rheumatism. 2007;57(2):352-4.

118. Nived O, Bengtsson AA, Jonsen A, Sturfelt G. Progressive multifocal leukoencephalopathy - the importance of early diagnosis illustrated in four cases. Lupus. 2008;17(11):1036-41.

119. Shprecher D, Frech T, Chin S, Eskandari R, Steffens J. Progressive multifocal leucoencephalopathy associated with lupus and methotrexate overdose. Lupus. 2008;17(11):1029-32.

120. Beghi E, Carpio A, Forsgren L, Hesdorffer DC, Malmgren K, Sander JW, et al. Recommendation for a definition of acute symptomatic seizure. Epilepsia. 2010;51(4):671-5.

121. Gustafsson JT, Gunnarsson I, Kallberg H, Pettersson S, Zickert A, Vikerfors A, et al. Cigarette smoking, antiphospholipid antibodies and vascular events in Systemic Lupus Erythematosus. Annals of the rheumatic diseases. 2015;74(8):1537-43.

122. Thangarajh M, Masterman T, Rot U, Duvefelt K, Brynedal B, Karrenbauer VD, et al. Increased levels of APRIL (a proliferation-inducing ligand) mRNA in multiple sclerosis. Journal of neuroimmunology. 2005;167(1-2):210-4.

123. Khademi M, Illes Z, Gielen AW, Marta M, Takazawa N, Baecher-Allan C, et al. T Cell Ig- and mucin-domain-containing molecule-3 (TIM-3) and TIM-1 molecules are differentially expressed on human Th1 and Th2 cells and in cerebrospinal fluid-derived mononuclear cells in multiple sclerosis. Journal of immunology (Baltimore, Md : 1950). 2004;172(11):7169-76.

124. Iacobaeus E, Ryschkewitsch C, Gravell M, Khademi M, Wallstrom E, Olsson T, et al. Analysis of cerebrospinal fluid and cerebrospinal fluid cells from patients with multiple sclerosis for detection of JC virus DNA. Multiple sclerosis. 2009;15(1):28-35.

125. Elfaitouri A, Hammarin AL, Blomberg J. Quantitative real-time PCR assay for detection of human polyomavirus infection. Journal of virological methods. 2006;135(2):207-13.

126. Fan JB, Oliphant A, Shen R, Kermani BG, Garcia F, Gunderson KL, et al. Highly parallel SNP genotyping. Cold Spring Harbor symposia on quantitative biology. 2003;68:69-78.

127. Lundstrom E, Gustafsson JT, Jonsen A, Leonard D, Zickert A, Elvin K, et al. HLA-DRB1*04/*13 alleles are associated with vascular disease and antiphospholipid antibodies in systemic lupus erythematosus. Annals of the rheumatic diseases. 2013;72(6):1018-25.

128. Van Assche G, Van Ranst M, Sciot R, Dubois B, Vermeire S, Noman M, et al. Progressive multifocal leukoencephalopathy after natalizumab therapy for Crohn's disease. The New England journal of medicine. 2005;353(4):362-8.

129. Langer-Gould A, Atlas SW, Green AJ, Bollen AW, Pelletier D. Progressive multifocal leukoencephalopathy in a patient treated with natalizumab. The New England journal of medicine. 2005;353(4):375-81.

130. Henegar CE, Eudy AM, Kharat V, Hill DD, Bennett D, Haight B. Progressive multifocal leukoencephalopathy in patients with systemic lupus erythematosus: a systematic literature review. Lupus. 2016;25(6):617-26.

131. Mikdashi J, Handwerger B, Langenberg P, Miller M, Kittner S. Baseline disease activity, hyperlipidemia, and hypertension are predictive factors for ischemic stroke and stroke severity in systemic lupus erythematosus. Stroke; a journal of cerebral circulation. 2007;38(2):281-5.

132. Hanly JG. Neuropsychiatric lupus. Rheumatic diseases clinics of North America. 2005;31(2):273-98, vi.

133. Gonzalez-Duarte A, Cantu-Brito CG, Ruano-Calderon L, Garcia-Ramos G. Clinical description of seizures in patients with systemic lupus erythematosus. European neurology. 2008;59(6):320-3.

134. Giqni A, Pileri PV, Faedda R, Gallo P, Sini A, Satta AE, et al. Interleukin 1, Interleukin 6, Interleukin 10, and Tumor Necrosis Factor α in Active and Quiescent Systemic Lupus Erythematosus. Journal Investigative Medicine. 2014 Jun;62(5):825-9