Cognitive dysfunction in autoimmune diseases

Maria Boge Lauvsnes

Dissertation for the degree philosophiae doctor (PhD)
at the University of Bergen

2015

Dissertation date: 18.06.2015

Year: 2015

Title: Cognitive dysfunction in autoimmune diseases

Author: Maria Boge Lauvsnes

Print: AIT OSLO AS / University of Bergen

Scientific environment

The work of this thesis has been performed in the Research group of Clinical Immunology at Stavanger University Hospital (SUS) between 2009 and 2015. This group, led by Professor Roald Omdal originated in 2003 from a cooperation between the Clinical Immunology Unit and the Departments of Internal Medicine, -Neurology, -Neuropsychology, -Radiology, -Neurophysiology and -Biochemistry at SUS. The research group collaborates with both national and international research partners.

In working with this thesis I have had extensive help and cooperation with Mona Beyer, radiologist and PhD at the Department of Radiology and Nuclear Medicine, Oslo University Hospital, National Hospital, professor Jan Terje Kvaløy, statistician at the Department of Mathematics and Natural Sciences, University of Stavanger, and professor Simone Appenzeller, rheumatologist at the Rheumatology Division, Department of Medicine, State University of Campinas, Brazil.

I have been associated as a research fellow to the Department of Internal Medicine, SUS and Department of Clinical Science at University of Bergen (UiB). My supervisor, Roald Omdal, is professor at Department of Clinical Science, UiB.

I have received financial support as a doctoral research fellow from the Western Norway Regional Health Authorities (Grant number 911453).

Abbreviations

ACR	American College of Rheumatology
AECG	American-European Consensus Group
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANA	Anti-nuclear antibodies
Anti-P	Anti-ribosomal P-protein antibodies
aPL	Anti-phospholipid antibodies
AQP4	Aquaporin 4
BBB	Blood brain barrier
BCR	B-cell receptor
BDI	Beck Depression Inventory
CI	Confidence interval
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computer tomography
DNA	Deoxyribonucleic acid
e.g.	for example
ELISA	Enzyme-linked immunosorbent assay
FDR	False discovery rate
FLAIR	Fluid Attenuated Inversion Recovery

FWE	Family-wise error
FWHM	Full-width-half-maximum
GM	Grey matter
HLA	Human leucocyte antigen
i.e.	Id est
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LPS	Lipopolysaccharide
MHC	Major histocompatibility complex
miRNA	MicroRNA
MRI	Magnetic resonance imaging
NK	Natural killer
NMDA	N-methyl-D-Aspartate
NMO	Neuromyelitis optica
NP	Neuropsychiatric
NPSLE	Neuropsychiatric systemic lupus erythematosus
PRRs	Pattern recognition receptors
pSS	Primary Sjögren's syndrome
R	Receptor

ROI	Region of interest
RS	Rasmussen's syndrome
SD	Standard Deviation
SSA	Sjögren’s syndrome A antigen
SSB	Sjögren’s syndrome B antigen
SLE	Systemic lupus erythematosus
SNPs	Single nucleotide polymorphisms
SUS	Stavanger University Hospital
T	Tesla
TCR	T-cell receptor
TE	Echo time
TFE	Turbo Field Echo
Th	T helper
TIV	Total intracranial volume
TPT	Tactual performance test
TR	Repetition time
Treg	Regulatory T cells
UIB	University of Bergen
VBM	Voxel-based morphometry
WM	White matter

WMH	White matter hyperintensities
WMS-R	Wechsler Memory Scale-Revised

Abstract

Background

Cerebral manifestations are common in autoimmune diseases. In systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS) cognitive dysfunction is frequently reported, affecting up to 50% of the patients in population-based studies. Several pathogenetic mechanisms for cerebral involvement are operative, including disturbances of cerebral circulation and neuro-reactive autoantibodies.

Anti-NR2 antibodies represent one of the players, and bind to the NR2 subtype of N-methyl-D-aspartate receptors. These are glutamate receptors that are abundant in hippocampus and are essential for memory formation. Anti-NR2 antibodies cause neuronal death in hippocampus and amygdala through excitotoxicity, followed by memory impairment and emotional disturbances.

It is common to observe global and/or regional atrophy as well as hyperintense white matter (WM) lesions in patients with SLE; but it is unclear whether these changes appear more frequently in patients with pSS than in healthy subjects.

Main objectives

- Summarize knowledge about the effect of anti-NR2 antibodies in animal models and in patients with SLE.
- Investigate whether presence of anti-NR2 antibodies influence cognitive function in patients with pSS and whether pSS patients have smaller hippocampi than healthy control subjects.
- Explore whether anti-NR2 antibodies are associated with hippocampal atrophy in patients with pSS and/or SLE.
- Explore whether patients with pSS have global or localized loss of brain matter compared to healthy subjects and investigate whether the total grey matter (GM) and WM volumes are related to clinical variables and/or disease factors.

Subjects and methods

All patients who received a diagnosis of SLE or pSS at Stavanger University Hospital between 1980 and 2004 were invited to participate in the study. Eighty-six SLE patients fulfilling the American College of Rheumatology classification criteria and 99 pSS patients fulfilling the American-European Consensus Group classification criteria were identified. Of these, 68 SLE- and 72 pSS patients gave informed consent to participate. 106 healthy control subjects were recruited among friends and neighbours of the patients, hospital staff and friends and neighbours of hospital staff.

All study participants were extensively examined by specialists in internal medicine and neurology, and cerebral MRI scan was performed. Cognitive function was evaluated by a specialist in neuropsychology and lumbar puncture was performed in all patients who accepted this procedure.

Anti-NR2 antibodies in serum and cerebrospinal fluid (CSF) were assessed by ELISA and electrochemoluminescence, respectively.

MRI analyses were performed by applying the SPM8 software and extensions to that.

Results

- Presence of anti-NR2 antibodies was in serum associated with worse performance in six out of ten, and in CSF with worse performance in eight out of ten memory tests, respectively
- A higher proportion of pSS patients with mental depression had anti-NR2 antibodies detectable in serum than pSS patients without depression
- pSS patients had smaller hippocampi than age- and gender matched healthy control subjects. Within the group of pSS patients, no differences in total hippocampal sizes between patients with and without anti-NR2 antibodies were revealed when corrections were made for age and gender
- In the combined cohort of the SLE- and the pSS patients, the patients with anti-NR2 antibodies in CSF had less hippocampal GM in voxelwise comparisons with patients without anti-NR2 antibodies. There were no localized differences

in GM volumes between SLE- and pSS patients, and no effects of anti-phospholipid antibodies, disease duration or present use of corticosteroids on hippocampal size were revealed

- pSS patients had lower global WM volumes than age- and gender matched healthy control subjects, but no localized atrophy. There were no global or localized differences in GM between the groups. Only gender influenced WM and GM volumes, no effects of tested disease characteristics or other variables could be seen

Conclusions

This work supports the hypothesis that anti-NR2 antibodies cause cognitive dysfunction, mental depression and can induce hippocampal neuronal death. Patients with pSS have less cerebral WM than matched healthy control subjects, suggesting that disease-related processes exert a deleterious effect on WM.

List of publications

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

Lauvsnes MB, Maroni SS, Appenzeller S, Beyer MK, Greve OJ, Kvaløy JT, Harboe E, Gøransson LG, Tjensvoll AB, Omdal R. Memory dysfunction in primary Sjogren's syndrome is associated with anti-NR2 antibodies. Arthritis Rheum. 2013;65(12):3209-17. Erratum in: Arthritis Rheumatol. 2014;66(4):989.

Lauvsnes MB, Beyer MK, Kvaløy JT, Greve OJ, Appenzeller S, Kvivik I, Harboe E, Tjensvoll AB, Gøransson LG, Omdal R. Association of Hippocampal Atrophy With Cerebrospinal Fluid Antibodies Against the NR2 Subtype of the N-Methyl-D-Aspartate Receptor in Patients With Systemic Lupus Erythematosus and Patients With Primary Sjogren's Syndrome. Arthritis Rheumatol. 2014;66(12):3387-94.

Lauvsnes MB, Beyer MK, Appenzeller S, Greve OJ, Harboe E, Gøransson LG, Tjensvoll AB, Omdal R. Loss of cerebral white matter in primary Sjogren's syndrome: a controlled volumetric magnetic resonance imaging study. Eur J Neurol. 2014;21(10):1324-9.

Contents

1. Background

1.1 The immune system

The immune system is commonly divided into the immediately acting "innate" and the slower, but more specifically acting "adaptive" immune system, the two parts being closely integrated and interdependent.

The innate immune system is the evolutionary oldest and represents "first line defence" against pathogens. It consists of physical and biochemical barriers, phagocytic cells and natural killer (NK) cells, cytokines, the complement system, acute phase proteins and other proteins. Pathogens are rapidly detected by pattern recognition receptors (PRRs) on innate immunity cells that recognize highly conserved molecular structures on pathogens, for instance peptidoglycans in Gram positive bacteria (1). Innate immune responses include (2):

- phagocytosis and intracellular killing of pathogens that are recognized by PRRs, complement receptors, or receptors for the constant part of immunoglobulins, i.e. Fc receptors
- killing by NK cells, antimicrobial peptides, proteolytic enzymes or complement membrane attack complex
- chemokine and cytokine production important for recruitment of other phagocytizing cells and immune regulation
- antigen presentation for cells of the adaptive immune system

In the adaptive immune system the B- and T-cells have specific receptors that recognize unique and specific antigens. Somatic recombination of DNA segments coding for the antigen binding region of the receptor result in an enormous repertoire of T and B lymphocyte receptors (TCR and BCR, respectively) (3). Given the vast recombination of genes, also T- and B lymphocytes with reactivity against “self” will develop. Thus, before the cells leave the primary lymphoid organs they will undergo negative selection by which autoreactive lymphocytes are deleted or lose their ability to respond to antigen binding. In addition to TCR and BCR the lymphocytes express

different co-receptors, cytokine receptors, as well as PRRs, all essential in further maturation into effector cells (4).

Naïve T- and B cells reside in secondary lymphoid organs (e.g. lymph nodes, mucosa associated lymphoid tissues) waiting for "their" antigen. Detection and binding of the specific antigen start the process where the inactive cells mature into effector cells and start clonal expansion, given the right microenvironment with supporting cells bearing essential co-receptors and stimulating cytokines (5).

T-lymphocytes recognize processed peptide antigens presented on major histocompatibility complexes (MHC). They are broadly divided into CD8+ cytotoxic T cells recognizing MHC I/peptide complex and CD4+ T cells recognizing MHC II/peptide complexes. MHC I is constitutively expressed on the cell-membrane of all nucleated cells, where it presents peptide sequences from intracellular proteins that have become “cut-up” in the proteasome. If non-self-peptides – for example viruses - are detected, the cytotoxic T cells induce apoptotic cell death of the antigen-presenting cell. The MHC II expression is limited to the professional antigen-presenting cells, e.g. B-cells, macrophages, monocytes and dendritic cells, which present processed protein fragments. Depending on the cytokine milieu, CD4+ T cells can develop into four subtypes, with different and specialized effector responses (5-8):

- T helper (Th)-1 cells mainly producing interferon (IFN)-γ and interleukin (IL)-2, both activating macrophages and cytotoxic T-cells essential for defence against intracellular pathogens
- Th-2 cells mainly producing IL-4, -5 and -6 and stimulating B cell antibody production
- Th-17 cells producing the pro-inflammatory cytokine IL-17 important for defence against extracellular pathogens
- Regulatory T cells (Treg) suppressing effector T cells by several mechanisms, including production of inhibitory cytokines like IL-10 and -35 and inducing cytolysis of both cytotoxic- and Th cells, as well as dendritic cells

Activated B-cells mature into antibody producing plasma cells. Antibodies are structurally similar to the membrane bound BCR and have several important functions;

they mark antigens for phagocytosis, can activate precursors of the complement system and neutralize antigens (5).

Both T- and B-cell responses require activation of different co-stimulatory receptors in addition to binding their specific antigen, and these co-receptors can be up- and down regulated in order to fine-tune and eventually turn off the immune response. A special feature of the adaptive immune system is that some of the activated B- and T-cells develop into long-lived memory cells that give a faster and stronger response if a second infection by the same antigen is encountered (3).

1.2 Autoimmunity

Despite the deletion and inactivation of autoreactive T- and B cells in the primary lymphoid organs, and the complex regulation of the immune response, immune reactions directed against self-antigens may occur in the predisposed individual, and prolonged inflammatory responses directed against self can thus cause extensive tissue damage. Autoreactive T-cells can mediate cytolysis and produce inflammatory cytokines; autoantibodies can mediate tissue damage through immune complex formation, induce phagocytosis of antibody-tagged cells, and interfere with normal cell functions through binding to cell surface receptors, like in Graves' disease and myasthenia gravis. During lifetime 5 – 10% of humans will develop autoimmune diseases (9). This includes a wide spectrum of conditions; some of them organ-specific like type 1 diabetes mellitus and autoimmune thyroiditis, while others involve multiple organs, i.e. the systemic autoimmune diseases.

The aetiology of autoimmune diseases is multifactorial with genetic factors playing major roles (10, 11). Mutations in a single gene may cause a dramatic increased risk for autoimmune disease, such as homozygote deficiency of C1q and C1r/C1s complement proteins, in which subjects have a 90% risk of developing systemic lupus erythematosus (SLE) (12). However, such mutations are rare. Most genetic variance between individuals are due to minor variations in the gene sequence, single-nucleotide polymorphisms (SNPs) being most common (13). SNPs are changes in the

DNA-sequence involving changes in only one base-pair, where both variants have a frequency > 1% in the population. If a SNP falls within the coding regions of genes, it may change the sequence or composition of amino acids in the gene product. Genetic susceptibility to autoimmunity are commonly thought to be modulated by stochastic combinations of variants of genes; mainly in MHC-coding regions, but also in other regions involved in innate and adaptive immune regulation mechanisms, leading to disturbed function of these (14, 15).

The 24% concordance rate of SLE in monozygotic twins illustrates that also other factors than genomic variance are of importance (16). Environmental factors can induce chemical DNA modifications (e.g. DNA methylations and histone modifications) that modify gene transcription without altering the base-pair sequence (17). These DNA modifications are commonly called epigenetic changes (17). Cigarette smoking and sun exposure are examples of environmental factors that can cause epigenetic changes, and lead to increased risk of autoimmunity (18). Also, a number of drugs may cause autoimmunity through epigenetic effects (17). Other epigenetic mechanisms are modulation of gene expression by microRNA (miRNA), small endogenous RNA sequences that downregulate translation by binding to messenger RNA (19). Up- or down-regulated expression of miRNAs that may influence lymphocyte co-receptor expression and cytokine production have been observed in SLE patients, and animal experiments indicate that miRNAs may play important roles in SLE pathogenesis (19).

Most autoimmune diseases have a much higher prevalence in females than in males. Sex hormones influence immune cell development via specific receptors on the cell surface. Oestrogen stimulates the production of B-cell stimulating cytokines (IL-4, IL-5, IL-6 and IL-10) from Th2 cells, and through this increases survival of autoreactive B cells (20). In addition, X chromosome abnormalities including skewed X chromosome inactivation, X duplication, X monosomy and X trisomy as well as translocation or epigenetic changes of specific gene sequences, are reported in autoimmune diseases and may contribute to the increased susceptibility observed in females (21).

Autoimmunity can arise during infections in which antibodies against the pathogen cross-react with self-antigens sharing high structural similarity with the pathogen. Also, autoreactive lymphocytes may arise due to the polyclonal activation that occurs during infections, and previously hidden self-antigens can be exposed as a consequence of tissue damage. Further, drugs can bind to the cell membrane and generate neo-antigens; for example penicillin and cephalosporin that cause haemolytic anaemia (22). Once autoreactive cells are activated in the predisposed subject, the complex interactions between the different cells and humoral components of the immune system may create a vicious circle where an increasing number of autoreactive cells find their previously cryptic self-antigens as a result of tissue damage, leaving the initiating event unidentified.

1.2.1 Systemic lupus erythematosus

SLE is often considered as the prototype of autoimmune diseases. Immunological abnormalities in SLE patients are observed both in the innate and the adaptive immune system. A variety of autoantibodies are produced, including antibodies against double-stranded DNA, considered a hallmark of the disease. Formation of immune complexes and complement activation may cause inflammation and damage organs. Common clinical presentations of SLE include arthritis, dermatitis, glomerulonephritis, neurological manifestations, serositis, blood cytopenias, and thrombosis (23). The American College of Rheumatology (ACR) classification criteria for SLE are widely used for research purposes, and they are to some degree also used as diagnostic criteria (24). The prevalence of SLE in Scandinavian countries is 46-85 per 100,000 inhabitants; women affected almost 10 times more often than men, and with an observed median age at diagnosis of 47 years (25-27).

Involvement of the peripheral and the central nervous system (CNS) are reported in up to 50% of the patients (28-30), and are collectively named “neuropsychiatric SLE” (NPSLE). This implies that manifestations may be of neurologic as well as psychiatric origin. The reported prevalences of NPSLE vary greatly, probably due to different criteria used to establish such diagnoses.

CNS involvement may be diffuse like in cases with headache or depression, or focal like in stroke. To facilitate research ACR defined 19 NPSLE syndromes in 1999, proposed case definitions for the syndromes, and included algorithms for diagnoses and exclusion criteria (31).

1.2.2 Primary Sjögren's Syndrome (pSS)

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease histopathologically characterized by chronic inflammation and lymphocytic infiltration of exocrine glands, and immunologically by B cell activation and presence of anti-SSA- and anti-SSB antibodies (32). Classical symptoms are dry mouth and eyes due to disturbed glandular function, but also joint and muscular pain, fatigue and other general symptoms are commonly experienced. The clinical characteristics of Sjögren's syndrome may also present concomitant with other autoimmune diseases, e.g. SLE or rheumatoid arthritis; in these cases it is termed secondary Sjögren's syndrome.

pSS affects mainly women, with highest incidence between 40 and 60 years of age (32). The prevalence in the general population in Norway was recently found to be 50 per 100,000 (33).

A number of different classification criteria for pSS have been proposed and used over time, varying in the demand for subjective symptoms, signs, or other items or manifestations to be fulfilled. Today the American-European Consensus Group (AECG) criteria (34) are widely accepted and used although proposals for new and more objective and stringent criteria have been published (35).

As in SLE, both the peripheral- and the CNS may be involved in pSS, however, with large variations in reported prevalences (36). There are several possible explanations for this variance, the most obvious being bias in recruitment; patients examined in neurological departments will tend to have more neurological involvement than patients from population-based cohorts. Also tertiary hospitals tend to have more complicated cases than community-based health services. Further, use of different classification criteria and lack of agreement upon which manifestations to include

(confer the ACR case definition on NPSLE) may influence this. Finally, differences in neurological involvement may be due to genetic and environmental factors. Applying the AECG criteria in a close-to population based cohort we found that most pSS patients had at least one neuropsychiatric manifestation; headache, peripheral neuropathy and cognitive dysfunction being the most common (30).

1.3 The brain and its protection

Largely, the brain tissue is composed of neurons with their dendrites and axons, glia cells and capillaries, and is divided into grey matter (GM) and white matter (WM). GM mainly comprises neuronal cell bodies and glia cells with sparse myelinated axons and is located in the cerebral and cerebellar cortex as well as in deep nuclei in cerebrum, cerebellum and the brain stem. WM on the other hand mainly comprises myelinated axons and glia cells and few neuronal cell bodies (37). The glia cells include oligodendrocytes that form the myelin sheets surrounding the neuronal axons, astrocytes that through different mechanisms maintain homeostasis (e.g. being part of the blood-brain barrier [BBB], providing nutrients to neurons, regulating blood flow and recycling neurotransmitters), and microglia that are brain residing macrophages (37). The brain is surrounded by cerebrospinal fluid (CSF), mainly produced by ependymal cells in the central ventricles of the brain, while a small proportion results from drainage of brain interstitial fluid (38, 39). The exchange of soluble molecules and cells between the blood and brain is tightly regulated by the BBB. The BBB comprises endothelial cells connected with tight junctions without intracellular fenestration, the capillary basement membrane and astrocytes (40). The passive exchange of molecules through the BBB is highly restricted, but there are several mechanisms for controlled transfer of specific molecules and substances. These transport mechanisms can be up- or down regulated according to the requirements of the CNS (40). There is a constant communication between neurons, glial cells and the endothelial cells, as well as the peripheral immune cells. This crosstalk is mediated through neurotransmission, cytokines and vasoactive substances (41). Activated T-cells from peripheral blood are able to pass the BBB into the CSF where they are

presented to antigens by meningeal and perivascular macrophages (42). In rats intrathecal antibody production can occur in the presence of an intact BBB (43). This indicates that activated B-cells are able to pass the BBB and in presence of "its" antigen and T-cell help, develop into antibody producing plasma cells (43). In humans, presence of "oligoclonal bands" in CSF (not corresponding with findings in blood) and increased IgG indexes are signs of intrathecal antibody production. Oligoclonal bands are narrow IgG bands visualized by electrophoresis/isoelectric focusing, and represent antibodies arising from specific clones (44). The IgG index is calculated as a ratio of (CSF IgG / CSF albumin) to (serum IgG / serum albumin). Albumin is not produced in the CNS, thus the CSF albumin to serum albumin ratio (Q-albumin) reflects the BBB permeability (44). Disruption of the BBB may occur during infections, smoking and hypertensive states (45-47).

1.4 Cognition and cognitive dysfunction

Cognition relates to the mental processes involved in acquisition of information, understanding, learning, remembering, decision making and problem solving. These processes of generating adequate responses to a given environment are the essential functions of the brain, and the mechanisms have engaged researchers for centuries. Early autopsy studies and observation of clinical consequences of cerebral lesions as well as more recent imaging techniques (like functional magnetic resonance imaging [MRI] and diffusion tensor imaging) have resulted in an understanding that cognitive functions involve widely distributed neuronal networks (i.e. cortical areas and neuronal fibres connecting these) (48).

Cognitive dysfunction is one of the NPSLE manifestations proposed by the ACR, and with the following diagnostic criteria (49):

- Documented impairment in one or more of the cognitive domains (simple attention, complex attention, memory, visuospatial processing, language, reasoning/problem solving, psychomotor speed, executive functions)
- The deficits represent a significant decline from a former level of functioning (if known)

- The cognitive deficits may cause varying degrees of impairment in social, educational or occupational functioning

The reported prevalence of cognitive dysfunction in SLE patients varies; some of this variance could be explained by differences in patient recruitment. Further, differences in neuropsychological testing probably add to this variation for example whether systematically neuropsychological testing are performed, the selection of tests applied, which population define the normative test score and the cut-off set for impairment. In an early study in which systematic neuropsychological assessment were performed in consecutive in- and outpatients, cognitive dysfunctions were found in 40-80%; the highest prevalence in patients with prior major NP involvement (e.g. hemiparesis, seizure) (50). In our population-based cohort we revealed cognitive impairment in 46% of the patients; 22% with mild and 24% with moderate to severe impairment (30). In another Nordic population-based SLE cohort, cognitive dysfunction was reported in 81% of the patients (28). However, the impairment was considered mild in 70%, giving a 24% prevalence of moderate to severe cognitive impairment. The cognitive dysfunction in SLE patients seems to fluctuate over time, is in general not progressive, and improvement may occur (51-53). All cognitive domains can be affected and there is not a "SLE-specific" pattern.

Studies in pSS patients in which systematic neuropsychological assessments have been performed are rare. In three such studies cognitive dysfunction were reported in 42-50% of the patients (30, 54, 55). Others have reported prevalences as low as 22% (56) and as high as 80% (57).

There is only one longitudinal study assessing cognitive function in pSS patients (58). This study concluded that cognitive dysfunction did not seem to be progressive over time.

1.5 Mechanisms for cerebral involvement in SLE and pSS

Cerebral manifestations contribute both to morbidity and mortality in SLE. Given the broad repertoire of NP manifestations in patients with SLE and pSS, it is likely that more than one mechanism is operative, and it is also likely that different mechanisms may act simultaneously in the individual patient. As SLE and pSS share clinical, immunological and genetic features, some of the mechanisms for NP involvement in these two diseases may be the same.

Only a few systematic autopsy studies have been performed in SLE patients. In these studies microinfarcts, microhaemorrhages, and small vessel vasculopathy have been common findings in the brain. Also, cortical atrophy, larger infarcts, haemorrhages, and patchy demyelination occur, while true vasculitis is rare (59-62). Autopsy studies in patients with pSS are even more rare than in SLE. One study described three patients; otherwise only a few case reports exist (63-65). As in SLE, microinfarcts, microhaemorrhages and small vessel vasculopathy are evident. Further, multifocal WM lesions composed of myelin pallor with diffuse margins, axonal swellings and perivascular lymphocytic infiltrates were described in one case report (65). Lymphocytic infiltrates in the leptomeninges have been seen, but no inflammatory cell infiltration of the brain parenchyma (64, 66). Histologically verified cerebral vasculitis has not been observed (64-66).

Generally, increased concentrations of glial fibrillary acidic protein and neurofilament triplet protein in the CSF are regarded as signs of neuronal and glia cell injury. In one SLE study such findings were observed in patients with NP involvement (67). Also, CSF pleocytosis, oligoclonale immunoglobulin bands and increased IgG indexes occur in 30-45% of both SLE and pSS patients, however not necessarily in patients with NP manifestations (44, 66-69).

A variety of autoantibodies could potentially bind to small cerebral blood vessel endothelium, and neuronal- as well as glial cellular elements and lead to cerebral neuronal disturbances in the patients. Such mechanisms could include both thrombotic

microangiopathy, thromboembolism in larger vessels, and activation or inhibition of neuronal receptors.

There is a well-known association in SLE patients between anti-phospholipid antibodies (aPL) and thromboembolism – *the anti-phospholipid syndrome* (70). Stroke occur in 15% of SLE patients (71), while it occurs in only 1.5-3% in pSS patients (30, 72) The considerably lower prevalence observed in pSS patients is therefore probably due to aPL being more rare in these patients (70, 73, 74). However, it is interesting that SLE patients without cerebral strokes, but with aPL may present with disturbed cognitive function (75, 76). This indicates that aPL can modify neuronal function by influencing the cerebral microcirculation and give rise to more subtle cerebral impairments. Dysfunctional cells that constitute the BBB could lead to suboptimal barrier integrity and leakage of neuroreactive antibodies into the brain. Also, aPL could cause neuronal dysfunction by binding to yet unknown antigens.

Anti-ribosomal P-protein antibodies (anti-P) recognize specific proteins on the ribosomes. In some studies SLE psychosis have been reported more frequently in subjects with anti-P detected in blood compared to SLE patients without anti-P (77, 78), while others have not been able to confirm this (79). The presence of anti-P in CSF has been postulated to cause several NPSLE manifestations (80, 81). The clinical relevance of anti-P is strengthened by animal studies that provide a potential mechanism for neuronal disturbances: P antigens have been discovered on the neuronal cell membranes of rats and mice, especially in the hippocampus, and injection of anti-P into rat brains resulted in neuronal apoptosis (82). In a recent murine study in which the BBB was abrogated by LPS, circulating anti-P caused memory impairment, while direct injection into the hippocampus caused hippocampal atrophy, indicating there may be a dose-dependent effect (83). Anti-P also occur in pSS patients (84). However, in one study that aimed to investigate the possible role of anti-P for NP in pSS, no patients with anti-P in serum were discovered (85).

Anti-neuronal antibodies have for decades been associated with NP manifestations in SLE patients, although no specific targets or pathogenetic mechanisms are known (86,

87). No roles for anti-neuronal antibodies in NP manifestations of pSS patients have been revealed (85).

Anti-SSA antibodies, also known as anti-SSA/Ro antibodies, constitute one of several antinuclear antibody (ANA) subtypes (88). Anti-SSA antibodies recognize either of two different ribonucleoprotein epitopes, Ro-52 or Ro-60 (88). The Ro-52 and Ro-60 proteins show different cellular localization and function. While Ro-60 identifies misfolded RNAs and marks them for degradation in the proteasome, Ro-52 is an ubiquitin ligase also marking proteins for proteosomal degradation (88). Presence of SSA antibodies is included in the AECG classification criteria for pSS and more than 50% of pSS patients harbour these antibodies (32). The concentration of anti-SSA antibodies in blood correlates with total gammaglobulin levels, as well as level of B-cell activating factor - BAFF (89). In a study including patients with primary- or secondary Sjögren's syndrome, more severe CNS involvement was evident in patients with anti-SSA antibodies. The authors suggested that anti-SSA antibodies could bind to endothelial cell peptides and disturb vascular function (90). However, in two later studies, no relations between anti-SSA antibodies and neurological manifestations were observed (66, 69).

Antibodies against aquaporin 4 (AQP4) cause neuromyelitis optica (NMO), a condition in which the optic nerve and spinal cord are selectively affected (91). This condition is characterized by demyelination and spinal cord GM necrosis. AQP4 on astrocytes play a critical role in water and ion transport in several parts of the brain, including neocortex, hippocampus, the optic nerve and spinal cord (92). One proposed mechanism is that binding of antibodies to AQP4 on astrocytes reduce the AQP4 expression with a subsequent down-regulation of the most important transporter for synaptic glutamate re-uptake, eventually causing neuronal and oligodendrocyte death by excitotoxicity (91). Anti-AQP4 antibodies have been observed both in SLE and in pSS patients with NMO (93).

The role of antibodies against *N*-methyl-D-aspartate (NMDA) receptors will be discussed in chapter 1.5.1.

It is important to have in mind that in patients with autoimmune diseases like SLE and pSS neuropsychiatric manifestations may occur concomitant and independent of the autoimmune disease itself. NP may be secondary to metabolic disturbances, uraemia, hypertension and infections; all phenomena occurring more frequently in e.g. SLE patients than in otherwise healthy subjects (94).

1.5.1 The role of NMDA receptor antibodies

1.5.1.1 The NMDA receptor

Glutamate is the main excitatory neurotransmitter, and membrane-bound receptors for glutamate are widespread throughout the brain and spinal cord. There are two main groups of glutamate receptors; first - metabotropic in which receptor activation leads to activation of transmembrane and intracellular proteins, and second - ionotropic receptors where ion channels open when the receptors are engaged. The NMDA receptor (NMDAR) is a subtype of the ionotropic glutamate receptor composed of two NR1 subunits in a complex with two of the four NR2 (a-d), or two NR3 (a-b) subunits, or a combination of an NR2 and NR3 subunit (95). The different subtypes are named based on subunit composition. The NMDAR function is mainly dependent of the subunits composition and the neuronal localization of the receptor (i.e synaptic versus extrasynaptic; the latter being more prone to activate pro-death pathways) (96). At birth only NR2b and NR2d are present, but during stimulation and development NR2a evolve in all parts of the brain, while the NR2d become rare and later are confined to the mesencephalon and diencephalon. NR2c are present in cerebellum and in the olfactory bulb. In contrast, NR2b occurrence continues, mainly in cortex and at a particularly high density in hippocampus (96, 97).

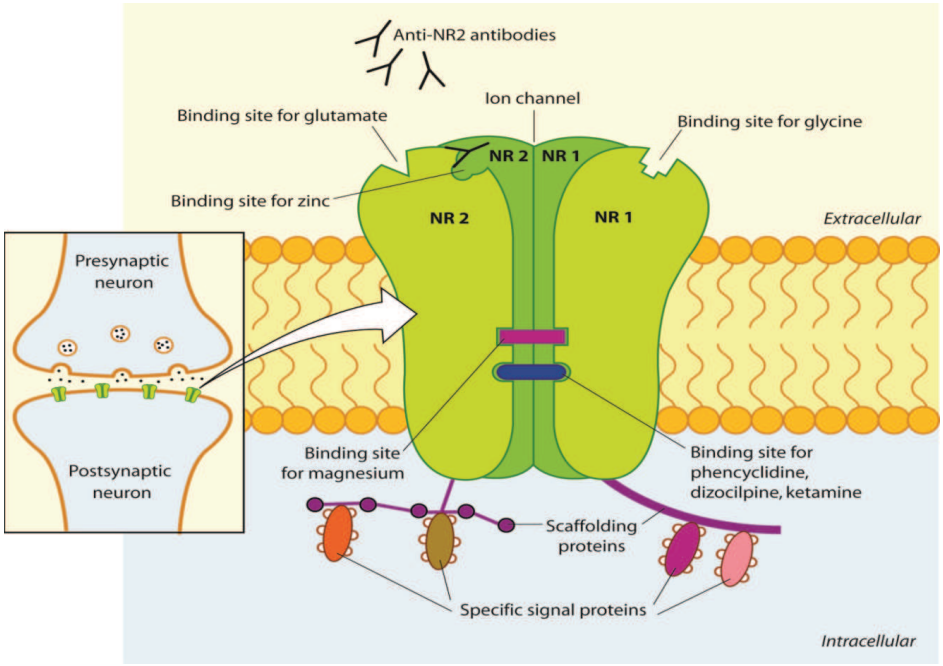

Figure 1 (98): The NMDA receptor forms an ion channel with high permeability to Ca^{2+} ions (97). The receptor has extracellular binding sites for the agonist glutamate and its essential co-agonist, glycine (97). In the resting state, the ion channel is blocked by Mg^{2+}. When Zn^{2+} ions bind to a site near the ion channel a voltage-independent block occurs that modifies the channel gating properties. Inside the ion channel several NMDAR antagonists can bind (99). The cytoplasmic tails of both the NR1 and NR2 subunits are linked to signalling proteins through different scaffolding proteins (100).

The NMDAR constitute an ion channel which in the resting state is blocked by Mg^{2+} (99). The receptor must be activated to open the channel, not only by binding of glutamate and the essential co-agonist glycine, but also by depolarization of the cell membrane. NR2a and NR2b receptors have an especially high demand of depolarization compared to NR2c and NR2d subunits for release of Mg^{2+} to occur. The ion channel allows calcium influx when open, this influx is particularly rapid in NR2a and NR2b receptors. Under physiological conditions, NMDAR responses mediate survival signals for developing neurons, and regulate synaptic strength and neuronal plasticity (101). The rapid NMDAR-gated Ca^{2+} influx can induce long-term potentiation, a fundamental process for learning and formation of memory in the

hippocampus (102). However, a sustained opening of the ion channel can lead to abnormally high intracellular Ca^{2+} levels, causing distorted neuronal function and eventually cell death by apoptosis (103). Thus, in addition to the complex receptor activation, there is a binding site for Zn^{2+} near the ion channel at NR2a and NR2b subunits, and binding of Zn^{2+} to this site cause a voltage-independent inhibition of the NMDARs (104).

NMDARs play important roles in acute and chronic neurologic and psychiatric disorders (105).

1.5.1.2 Experimental studies on anti-NR2 antibodies

Initially investigating origin and pathogenicity of anti-DNA autoantibodies, a research group lead by professor Diamond induced anti-DNA antibodies (R4a) which happened to display cross-reactivity with both the human- and the murine NR2a and NR2b subunits of the NMDAR (106). Based on the knowledge that cognitive dysfunction is common in SLE patients, and the NMDARs are of such importance in cognition, a series of experiments were performed to explore the potential clinical relevance of these antibodies. To summarize their main findings:

- When R4A was injected into mouse brains, the antibodies caused hippocampal neuronal loss by apoptosis without inflammation. Neuronal loss could be prevented by co-administration of the NMDAR antagonist MK-801. Hippocampal neuronal death also followed when CSF from a lupus patient with cognitive dysfunction was injected into mouse brains (107).
- Systemic anti-NR2 antibodies caused by immunization in mice did not give neuronal loss. However, when the BBB was abrogated by lipopolysaccharide (LPS), hippocampal neuronal loss accompanied by memory impairment was observed. The memory impairment was prevented by co-administration of memantine, another NMDAR antagonist (108).
- When epinephrine was used for BBB abrogation instead of LPS, anti-NR2 antibodies selectively bound to and caused death of amygdalar neurons and not of hippocampal. The amygdalar neuronal loss was followed by emotional disturbances. In addition to memantine, synthetic DWEYS-peptide could prevent neuron damage when administered together with epinephrine (109).

- Hippocampal loss and memory impairment could be induced in mice by giving serum from SLE patients with anti-NR2 antibodies intravenously followed by LPS. The same effect was seen when IgG eluted from brain tissue of a SLE patient with progressive, severe cognitive impairment was injected directly into mouse brains (110).
- In the brains of four NPSLE patients, endogenous IgG bound to hippocampal neurons was found; they collocated with marked NR2a- and NR2b antibodies (110).
- The effect of anti-NR2 antibodies is dose-dependent, at low concentration they alter neuronal synaptic function and at high concentration they cause apoptosis (111).

Further knowledge of the mechanism of these antibodies came from a study by Gono and colleagues. They observed in cell cultures that anti-NR2 antibodies bound specifically to the Zn^{2+} binding site of the NR2a or NR2b subunit, and in a dose-dependent manner blocked the inhibitory role of Zn^{2+}. This resulted in a prolonged opening of the ion channel when the receptor was activated, followed by increased and cytotoxic intracellular concentrations of Ca^{2+} (112).

Finally, NR2a and NR2b proteins are present on human cerebral endothelial cells and a recent study revealed that anti-NR2 antibodies bind to the endothelial cells and cause increased expression of adhesion molecules and release of proinflammatory cytokines from the endothelial cells. This mechanism could possibly be operative in vivo and be another cause for BBB disruption (113).

Altogether, there is substantial support for a role for anti-NR2 antibodies in the pathogenesis of cerebral NPSLE.

1.5.1.3 Anti-NR2 antibodies in SLE patients

Anti-NR2 antibodies in serum are reported in approximately 30% of SLE patients (114-118) and in 5-10% of patients with other autoimmune diseases (such as myasthenia gravis, autoimmune polyendocrine syndrome, rheumatoid arthritis, multiple sclerosis, polymyositis/dermatomyositis and systemic sclerosis) (116-118).

In the original murine studies anti-NR2 antibodies were cross-reactive with native DNA. Several later human studies demonstrate that not all anti-NR2 antibodies are

cross-reactive with DNA, but appear as unique NR2-reactive antibodies (116, 118, 119).

Although a common belief is that anti-NR2 antibodies are produced in the periphery and pass over the BBB into CSF, an alternative hypothesis could be that they are produced intrathecally by resident plasma cells. Both mechanisms could be operative in SLE patients (120).

It is known that anti-NR2 antibody levels in serum and CSF are correlated (119). In general, the anti-NR2 antibody concentration is higher in blood than in CSF (121, 122), but the opposite has been described in patients with acute cerebral NPSLE (122) and in complex NPSLE (119). SLE patients with septic meningitis reportedly have higher anti-NR2 antibody concentrations in CSF than SLE patients hospitalized for other reasons, conceivably as a result of a disruption of the BBB (122). In nine patients, a fall in intrathecal anti-NR2 antibody levels occurred after intensive corticosteroid treatment, possibly reflecting re-established BBB function (119). Associations between presence of anti-NR2 antibodies and NPSLE have been inconsistently reported. In six studies, cognitive function was systematically assessed by neuropsychological testing (114, 115, 117, 123-125); but only in one of these was it possible to demonstrate an association between anti-NR2 antibodies in serum and cognitive impairment (123). In two studies anti-NR2 antibodies and mental depression were associated (114, 123), while three other studies could not confirm such relations (78, 115, 125). None of these studies provided any information about anti-NR2 antibodies in the CSF.

Four studies investigated the possible implications of anti-NR2 antibodies being present in CSF. Associations between anti-NR2 antibodies in CSF and diffuse and complex NPSLE were observed in two studies (119, 121). In another SLE study, anti-NR2 antibodies in CSF were found more frequently in patients with CNS manifestations than those with peripheral nervous system manifestations (122). In a recent SLE study, higher anti-NR2 antibody concentrations were seen in patients with

acute confusional state, compared with patients with less severe and diffuse NP manifestations (120).

Four studies with repeated measures of anti-NR2 antibodies have been published (two reporting serum data and two CSF data). In three of these studies anti-NR2 antibody concentrations correlated with the severity of the NPSLE manifestations (116, 117, 119, 122).

1.5.1.4 Anti-NR2 antibodies in other conditions

Rasmussen's syndrome (RS) is a rare autoimmune syndrome, mainly affecting children, characterized by seizures, progressive hemiparesis, mental retardation and brain atrophy (126). It was originally thought to be caused by autoantibodies against the ionotropic glutamate receptor subtype α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), but it has later been clear that the condition also occurs in the absence of these antibodies. In a Japanese study of 20 patients with histologically verified (10) or clinically suspected (10) RS, all with verified, and nine out of ten with suspected RS, had anti-NR2 antibodies (127). Finally, in one study NMDAR antibodies have been reported in 10% of the patients with schizophrenia at the time of diagnosis (128).

1.5.1.5 Anti-NMDAR encephalitis

Anti-NMDAR encephalitis, a condition causing both psychiatric and neurologic signs and symptoms, is caused by autoantibodies directed against the NR1 subunit of NMDAR. The condition was first described as a paraneoplastic phenomenon secondary to ovarian teratoma. It is now well known that this condition constitute a genuine autoimmune disease not necessarily related to neoplastic diseases (129).

In rat studies, it has been demonstrated that the number of NMDARs on the neuronal cell surface are reduced as a consequence of internalization of the NMDARs upon binding of anti-NR1 antibodies. This effect is dose-dependent and the number of NMDARs returns to baseline when the antibodies are removed (130). In line with this, the disease phenomena fade as the concentration of anti-NR1 antibodies in CSF fall, and complete recovery is most commonly seen (129).

1.6 Findings in previous MRI studies of SLE and pSS patients

Cerebral imaging can reveal anatomical changes in both SLE and pSS patients. Computer tomography (CT) is fast and easily accessible and is commonly applied for diagnosis in acute neurological manifestations, especially strokes. Magnetic resonance imaging (MRI) has, however, much higher sensitivity to reveal changes in soft tissue, and is thus often preferred both in clinical settings and research. Abnormalities detected by MRI in SLE and pSS include hyperintense WM lesions (WMH), global and regional atrophy and cerebral infarcts. MRI abnormalities are not always reflected clinically, and conversely; clinical signs and symptoms are not always accompanied by MRI changes. It is more likely that patients suffering from focal rather than diffuse CNS manifestations display MRI abnormalities (131). In a study in which premortal MRI and postmortal histopathologic findings were compared, there was a 100% sensitivity for detecting large cerebral infarcts, while microinfarcts were infrequently detected by MRI (132). Important, there are no MRI abnormalities specific for SLE/pSS; such findings may be observed in otherwise healthy persons as well.

1.6.1 Cerebral strokes

Cerebral infarcts occur more frequently in patients with SLE than in pSS, in one study 12% vs 3%, respectively (30). In fatal NPSLE, cerebral infarction occurred in 29% of the patients and intracranial haemorrhages in 21% (132).

1.6.2 White matter hyperintensities

WMH are hyperintense signals detected in T2-weighted images. They appear with increasing frequency with increasing age in healthy subjects. Cerebrovascular risk factors (hypertension, diabetes mellitus or cardiac disorder) are cofactors that accelerate WMH development (133). WMH are the most frequent MRI finding in both SLE and pSS patients, and they appear earlier and in increased numbers in SLE compared to healthy subjects. It is unclear whether this is the case in pSS (54, 57, 134-136). Histopathological studies of WMH in SLE reveal acute microinfarcts and

microhaemorrhages with focal oedema in some cases, but also small resolved infarcts (132). Thus, WMH in SLE are dynamic lesions and though some of the lesions persist, even large lesions can resolve (135, 137). Increased number and size of WMH are associated with CNS involvement in both pSS and SLE patients; the specificity of predicting such involvement is however limited (66, 132). There are conflicting results regarding the significance of WMH on cognitive function, a possible cause of this discrepancy could be that a critical mass or localization affecting essential neuronal fibres are required for WMH to impact cognition (55, 138-141).

1.6.3 Global atrophy of the brain

Global atrophy of the brain is the second most common finding in SLE patients (135). It appears early; in one study atrophy was present in 20% of the patients when scanned within a year after diagnosed with SLE (142). Further, global atrophy was found in almost all children and adolescents in a study examining MRIs taken in a clinical setting of NPSLE. In this group the volume loss was moderate to severe in 25% (136). Cerebral atrophy is accelerated over time in SLE patients compared to healthy subjects (143, 144) and is more severe with longstanding disease, presence of aPL and corticosteroid treatment. These associations are, however, not consistently found (143, 145). Cerebral atrophy was found in 16-40% of pSS patients by visual inspection of MRI images (146-148), and also demonstrated in a study applying computer-based analyses (149). These findings contrast another computer-based volumetric study in which no GM atrophy was revealed (54). Cerebral atrophy has been associated with cognitive dysfunction in both children and adults with SLE, while these matters have not been explored in pSS (143, 145).

1.6.4 Regional atrophy

Progressive hippocampal atrophy has been described in adult SLE patients, and corpus callosum atrophy demonstrated in both children and adults with SLE. Atrophy of these brain structures were accompanied by cognitive impairment (136, 150, 151).

1.7 MRI volumetric analysis

There are several software packages used for computer-based volumetry. Statistical Parametric mapping (SPM; http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) is one of the most commonly applied. Before analyses can take place, it is necessary to *preprocess* the MRI images. Biases caused by inhomogeneity of the MRI scanner magnetic field are corrected and the images are normalized against a template to achieve a standard size and to be spatially aligned (152). Further, based on signal intensities and a priori probability maps, the images are segmented into GM, WM and CSF. The next step is modulation, where potential changes in brain volumes during the normalization step is corrected for by an accordingly change in signal intensity, e.g. if the volume of a region is halved by the normalization, the signal intensity is doubled in the modulation. Finally, the images are smoothed by a Gaussian kernel to obtain more normally distributed data and reduce potential errors occurring during the normalization (152).

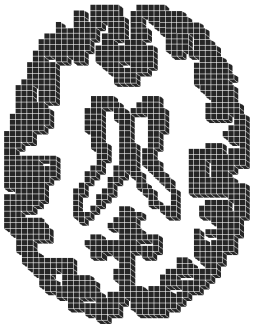

Figure 2: 3-dimensional MRI images are built up by voxels. This figure illustrates one layer of GM voxels. For illustration purposes the voxel size is much larger than applied in imaging analysis.

1.7.1 Global volume analyses

The SPM software can be used to estimate global volumes of both GM and WM, and by applying designated software extensions, volumes of specific regions of interest (ROI), for instance hippocampus, can be measured. For analyses of global or ROI

volumes the differences in volumes between groups must be large enough to turn out significant, but there is no requirement that volume differences between the groups are at the same space.

1.7.2 Voxel-wise analyses

When MRI images are spatially aligned and modulated, brain volumes of groups can be compared voxel by voxel for instance by *t*-tests in SPM. These tests can be performed across all GM or WM voxels, or across a specific ROI, depending on the hypothesis. The results are given as statistical parametric maps displaying clusters of voxels where signal intensities, i.e. volumes, differ between the groups.

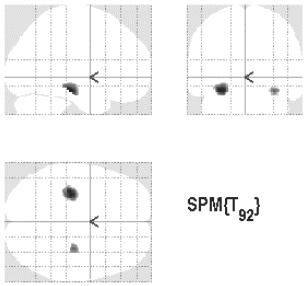

Figure 3: Statistical parametric map displaying areas where volumes differ between the tested groups in three planes

As each voxel is analysed separately, this voxelwise approach requires that volume differences have exact the same localization. The mass-univariate approach to these analyses, generate an extremely large number of tests, it is therefore necessary to correct for multiple testing to limit false positive results. Family-wise error (FWE) correction is a conservative and commonly applied method for such correction. The aim of this method is to limit the overall probability of getting false positive results to the set significance level, typically 0.05. The corrected *p*-value is thus 0.05 divided on total number of tests performed (153).

2. Aims of the study

- To summarize knowledge about the effects of anti-NR2 antibodies in animal models and in patients with SLE.
- To investigate whether anti-NR2 antibodies influence cognitive function in patients with pSS and whether pSS patients have smaller hippocampi than healthy control subjects
- To explore whether anti-NR2 antibodies influence the hippocampal sizes in patients with SLE and pSS
- To explore whether patients with pSS have diffuse (global) or a more localized loss of brain matter compared to healthy subjects, and investigate whether the total amount of GM and WM are related to clinical variables and/or disease factors.

3. Subjects and methods

3.1 Subjects

All SLE and pSS patients were recruited at Stavanger University Hospital (SUS), Norway. This is the only hospital in the southern part of Rogaland county, serving a population of 330,000 inhabitants at the time of examination.

3.1.1 Patients with SLE

Medical records of all patients given the diagnosis SLE between 1980 and 2004 were reviewed and 86 patients fulfilling the ACR revised classification criteria for SLE (24, 154) were identified. Sixty-eight (79%) of these patients gave written informed consent to participate in the study. Fifty-nine (87%) were females. Mean age was 43.8, standard deviation (SD) 13.6, and range 19.6 – 75.9 years.

3.1.2 Patients with pSS

The medical records were reviewed of (a) all patients given a diagnosis of pSS between 1980 and 2005, and (b) all patients who had a minor salivary gland biopsy performed between 1990 and 2004, and had a focus score $\geq$1(155). These biopsies were examined and scored at the Department of Pathology at SUS. Ninety-nine patients fulfilled the AECG criteria for pSS (34). Of these, 72 (73%) gave written informed consent to participate. Sixty-two (86%) were female. Mean age was 57.8, SD 13.0, and range 27.1 – 86.6 years.

3.1.3 Healthy subjects

106 age- (± 2 years) and gender-matched healthy subjects were recruited among unrelated friends and neighbours of the patients, hospital staff, and unrelated friends and neighbours of the hospital staff. Eighty-eight (83%) were female. Mean age was 50.6, SD 15.6, and range 21.2 – 88.1 years.

All study participants were Caucasian.

Details regarding subjects included and excluded in the different papers are given in Figure 4.

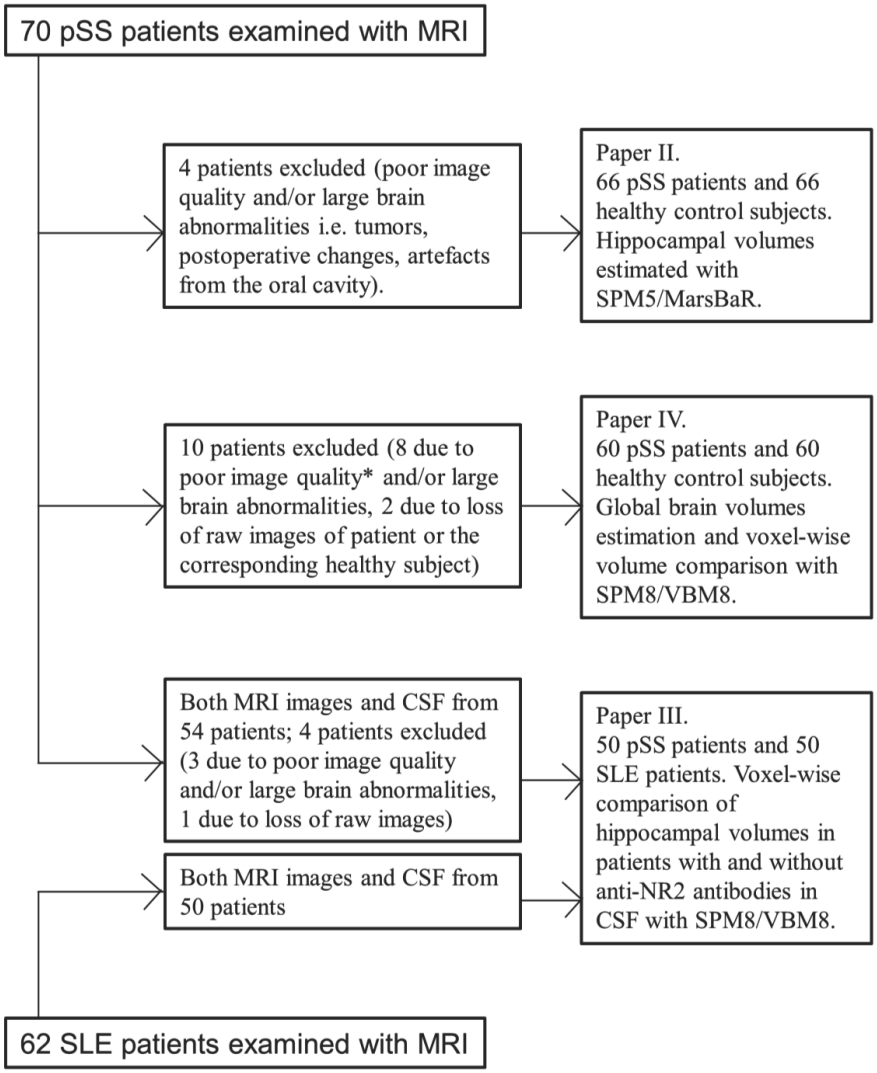

Figure 4: Selection of pSS- and SLE patients in our cohorts and study overview.
*Higher demands for image quality in whole-brain analyses than in hippocampus analyses, the latter only requires good definition of the hippocampus

3.2 Examination protocol

For research purposes only, all participants underwent extensive and standardized examination by specialists in internal medicine and neurology, and cerebral MRI scan was performed within a median of 12 days (range 0-75). In addition, all patients were subjected to neuropsychological assessment. Lumbar puncture was performed in all patients who accepted this procedure. The study was approved by the regional research ethics committee and was carried out in compliance with the Helsinki Declaration.

3.3 Neuropsychological assessment

The neuropsychological testing started at a fixed time in the morning; the tests were administered by a trained neuropsychological test technician and were completed within 3-4 hours. All tests were administered in a standardized manner, and the results were analysed by a clinical neuropsychologist.

Results from the neuropsychological assessment of the pSS patients were applied in Paper II. As the NMDAR function is of vital importance for learning and memory formation, we expected anti-NR2 antibodies to influence these functions, thus data from memory tests were selected. We used the sum of the weighted scores of four indices from the Wechsler Memory Scale-Revised (WMS-R) (156). WMS-R verbal memory and WMS-R visual memory provide composite measures of the immediate retrieval of verbal and visual material, respectively. WMS-R general memory provides a composite measure of the immediate retrieval of both verbal and visual material, and WMS-R delayed memory provides a composite measure of delayed retrieval of the same material after a 30-min interval. The Tactual Performance Test (TPT) (157) encompasses several cognitive domains (158) and performance is reported as the time to complete an assigned task using the dominant hand, the nondominant hand, both hands together and the total time to complete all tests. In relation to the baseline performance of the dominant hand, the TPT for the nondominant hand, the TPT for both hands together and TPT total time can be considered as tests that measure

learning. TPT location and TPT memory measure incidental tactile and spatial learning and retrieval, respectively.

3.4 Evaluation of mental depression

The Beck Depression Inventory (BDI) was used for evaluation of mood. A score ≥ 13 was regarded as reflecting clinical depression (159, 160).

3.5 Blood and CSF analyses

Routine haematological and biochemical tests were analysed in the hospital's laboratory. Screening for ANA was performed with the HEp-2000 assay (Immunoconcepts, Sacramento, CA, USA). The presence of anti-double-stranded (ds) DNA, anti-SSA, and anti-SSB antibodies was confirmed by ELISA with the QUANTA Lite ENA 6 assay (Inova Diagnostics, San Diego, CA, USA). Anti-dsDNA antibodies were also verified by Nova Lite dsDNA *Crithidia luciliae* 708200 indirect immunofluorescence assay (Inova Diagnostics). Testing for anti-cardiolipin IgM and IgG antibodies was performed with the QUANTA Lite ACA IgM and IgG ELISA (Inova Diagnostics). Lupus anticoagulant was screened for by measuring the activated partial thromboplastin time and dilute Russell's viper venom time (Dade Behring, Marburg, Germany). Anti-phospholipid antibodies (aPL) were considered positive if the patient had a positive anti-cardiolipin IgM- or IgG-antibody test, was lupus-anticoagulant positive, or any combination of these.

3.5.1 Anti-NR2 antibodies in blood

Serum anti-NR2 antibodies were analysed by an ELISA-based technique. An amidated and acetylated DWEYSVWLSN decapeptide was synthesized, purity was > 95% as assayed by high-performance liquid chromatography.

96-well microtiter plates (Greiner Bio-One, GmbH, Frickenhausen, Germany) were coated with 1 µg decapeptide in 100 µl phosphate buffered saline (PBS) (pH 7.4) in each well and left overnight at 4 °C. The wells were blocked with 10% foetal calf

serum (FCS) in PBS for 1 hour. The serum samples were diluted 1:50 with 10% FCS in PBS. The diluted samples were added in triplicate and incubated for 2 hours before bound serum antibodies were detected using Polyvalent Anti-Human IgGAM Peroxidase Conjugate (Sigma-Aldrich, St. Louis, MO, USA). Then o-phenylenediamine dihydrochloride (DakoCytomation, Glostrup, Denmark) was added as detection substrate, and after 30 minutes of incubation, the peroxidase reaction was stopped by adding 100 µL of 1 M H_2SO_4. Optical density (OD) was read at 492 nm with a Multiskan Ascent microplate photometer (Thermo Scientific, Waltham, MA, USA). Except for coating, all steps were conducted at room temperature and the plates were washed with PBS four times after each incubation.

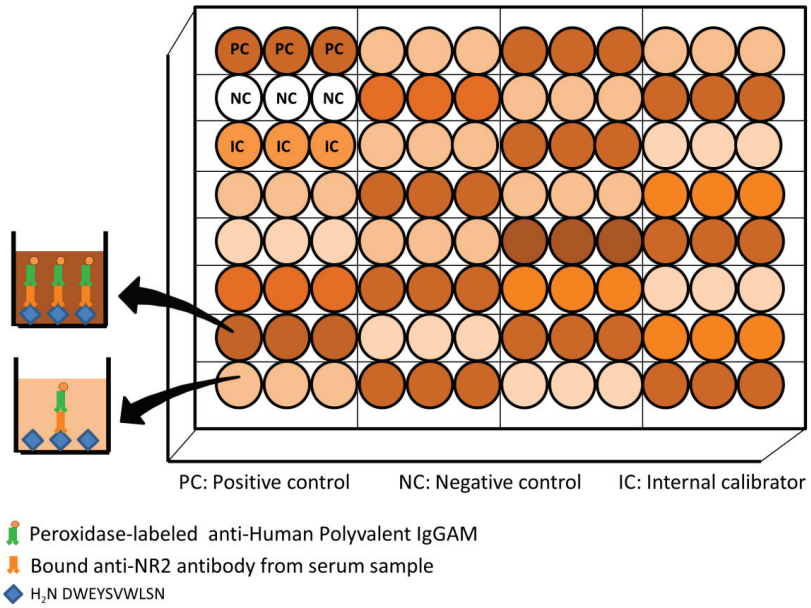

Figure 5: Triplicate serum samples from 29 subjects can be analysed on each plate in addition to the positive controls (PC), negative controls (NC) and the internal calibrators. The optical density increases with increasing peroxidase activity – i.e. increasing anti-NR2 antibody concentration, and is seen as a darker colour in the wells.

R4vg2b monoclonal antibody, kindly provided by Dr C.H. Kowal (Albert Einstein College of Medicine, NY, USA), was used as positive control, while 10% FCS in PBS served as negative control.

The analytical cut-off was based on the mean OD (0.46) + 3 SD (3 x 0.12) from 95 healthy blood donors. One patient showed an OD close to this value (0.86) and serum from this patient was used as an internal calibrator on all plates and this OD (0.86) also defined the cut-off value. The ratio against this cut-off was calculated for all samples; a ratio ≥ 1.0 was considered positive, and < 1.0 negative, for the presence of anti-NR2 antibodies.

3.5.2 Anti-NR2 antibodies in CSF

Anti-NR2 antibodies in CSF were measured by an electrochemiluminescence method on a SECTOR Imager 2400 platform (MSD, Gaithersburg, MD, USA). A high bind plate (L15XB-3; MSD) was coated with 25 µL of the synthetic DWEYSVWLSN decapeptide at a concentration of 2 µg/mL and incubated overnight at 4 °C. The plate was blocked with 150 µL of 3% bovine serum albumin (MSD Blocker A) for 1 hour. Then, 25 µL of each sample was added in duplicate to the wells and incubated for 2 hours. 25 µL (1 µg/mL) of anti-human antibody (goat) with Sulfo-TAG (MSD) was added and incubated for 1 hour. Read Buffer T (150 µL of 2x buffer; MSD) was added and results were read on a Sector Imager 2400 (MSD). With the exception of coating, all incubation steps were conducted at room temperature on a plate shaker (300-400 rpm), and the plate was thereafter washed three times with PBS (pH 7.2-7.3) plus 0.05% Tween 20.

The cut-off value for anti-NR2 antibodies was based on CSF from 24 subjects who underwent lumbar puncture as part of a neurological examination. None of these subjects later turned out to have any inflammatory, autoimmune, or malignant diseases. The CSF sample with the highest signal out of all these subjects (signal 13174 which was slightly higher than mean signal of all samples (5369) + 3 SD (3 x 2487) was chosen as the cut-off value/internal calibrator and measured together with the samples on each plate. For each sample, a ratio against the internal calibrator was

calculated. Samples with ratio ≥ 1.0 were considered positive, and < 1.0 were considered negative.

3.6 MRI

3.6.1 Image acquisition

The patients were examined using a 1.5-T Philips Gyroscan NT Intera Release 10 (Philips Medical Systems, Best, Netherlands).

MRI scanning protocol: Axial T2 turbo spin echo (SE) conducted with TR 3240 ms, TE 19 ms/80 ms, slices 5 mm, gap l.5 mm. Sagittal T2 fluid-attenuated inversion recovery (FLAIR) was performed with TR 6500 ms, inversion recovery 2200 ms, TE 105 ms, slices 5 mm, and gap 1 mm. Axial T1 3-dimensional turbo field echo (TFE) had a TR 17 ms, TE 4 ms, no gap. Field of view 230 x 230 mm, Matrix 256 x 256, nominal resolution 0.9 x 0.9 x 1.4 mm. Axial T1 SE (TR 525 ms, TE 12 ms, slices 5 mm, no gap) was performed before and after intravenous gadolinium contrast. Sagittal T1 SE was performed with TR 525 ms, TE 12 ms, slices 5 mm, and no gap. For the image analyses, axial T1 3-dimensional TFE with nominal resolution 0.9 x 0.9 x 1.4 mm were used.

MRI data for the SLE and pSS patients and healthy subjects were collected randomly to prevent collection biases like scanner calibration drifting over time (161).

3.6.2 Preprocessing

MRI images were pre-processed using default settings in the VBM8 extension (Gaser, http: //dbm.neuro.uni-jena.de/vbm/download/) of the SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). The images were bias-corrected, tissue classified (GM, WM and CSF) and registered by using linear and non-linear transformations within a unified model (162). The normalization included correction for individual brain size, thus we did not need to correct for total intracranial volume in our analyses. Finally, the images were smoothed using a 12-mm full-width-half-

maximum (FWHM) Gaussian Kernel to reduce the inter-subject variability and obtain a more normal distribution of the data (152).

The segmentation of GM and WM was visually examined for all study participants. Data quality and sample homogeneity were tested by viewing one normalized, unsegmented slice for all patients and healthy control subjects and evaluating a covariance matrix of the covariance among all volumes using the VBM8 tool (Gaser, http: //dbm.neuro.uni-jena.de/vbm/download/).

3.6.3 Global volume estimation (Paper IV)

Global GM, WM, and CSF volumes for the pSS patients and the corresponding healthy control subjects were calculated using the VBM8 tool. In addition, we calculated total intracranial volumes (TIV) as sums of GM, WM, and CSF volumes. TIV is considered to reflect premorbid brain size (161). Further, the following ratios were calculated; GM volume/TIV, WM volume/TIV and CSF volume/TIV.

3.6.4 Hippocampal volume estimation (Paper II)

The image preprocessing was performed with the VBM5 (Gaser, http://dbm.neuro.uni-jena.de/vbm/download/) extension to SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/). First, we created template images based on our healthy subject control group. Normalization and segmentation were performed with default settings. GM and WM were smoothed with an 11-mm full-width-half-maximum Gaussian Kernel. We used the MarsBar (www. marsbar.sourceforge.net) volume-of-interest analysis toolbox for SPM5 and selected right and left hippocampus region of interest by applying the "Automated anatomical labeling (aal) atlas" (www.gin.cnrs.fr/spip.php?article217). Estimation of hippocampus volumes was conducted by professor Simone Appenzeller.

3.6.5 Voxel-wise analyses (Papers III and IV)

The voxel-wise analyses were performed with the SMP8 software. An absolute mask was applied where all voxels with less than 10% probability of being GM or WM,

respectively, were excluded to avoid possible edge effects between different tissue types.

3.6.5.1 Analyses for Paper III

Disease duration, aPL and corticosteroid treatment have previously been reported to have a negative effect on hippocampal volumes in SLE patients (150). We first set up four models with independent-samples *t*-test design in which disease (SLE and pSS) was the grouping variable and either of anti-NR2 antibodies (Model 1), aPL, disease duration, or present use of corticosteroids were set as covariates. Age and gender were added as nuisance covariates in all models to exclude effects caused by these variables. Within a hippocampus mask we first tested for a main effect of group and the different covariates by two-way F contrasts. If an effect appeared, we continued with one-way *t*-contrasts to determine whether the variable had a positive or negative effect on hippocampal GM volumes. Further, a more complex model were constructed (Model 2), also with an independent-samples *t*-test design with disease (SLE and pSS) as the grouping variable and anti-NR2 antibodies, aPL, disease duration, and present use of corticosteroids as explanatory covariates and age and gender as nuisance covariates. This was done to evaluate the effect of anti-NR2 antibodies when controlled for the effect of the other covariates. We tested for interaction between group and presence of anti-NR2 antibodies in both Model 1 and 2. In model 2 we also tested for interaction between anti-NR2 antibodies and the other covariates.

We revealed an effect only from the presence of anti-NR2 antibodies on hippocampal GM volumes, and thus applied Model 1 for the final analyses where we explored whether anti-NR2 antibodies influenced GM volume in other parts of the brain by running *t*-tests across all GM voxels. Also in this whole-brain setting we tested for effect of disease groups and interaction between group and anti-NR2 antibodies. In these analyses, seven SLE and three pSS patients had to be excluded due to cortical infarcts or artefacts not involving the hippocampus.

FWE corrected $p < 0.05$ were set as the cluster significance threshold. However, as this was an exploratory study, we also reported all voxels where GM volume differed

between patients with and without anti-NR2 antibodies at a significance level of $p < 0.001$ uncorrected, although not all of them reached statistical significance after correction for multiple testing.

3.6.5.2 Analyses for Paper IV

For analyses of the groups of 60 pSS patients and 60 healthy control subjects we applied two samples *t*-tests to voxel-wise compare GM and WM volumes between the groups. We applied FWE corrected $p < 0.05$ as significance level on cluster level.

3.6.6 WMH-assessment

Two independent raters (MKB and OJG) assessed WMH according to the Scheltens visual rating scale (163). This is a semi-quantitative rating scale where the size and numbers of WMH within 4 anatomical regions are scored.

3.7 Statistical analyses

3.7.1 Paper II:

In the group of 66 pSS patients we wanted to establish models for memory test scores and hippocampal sizes and tested potential exploratory variables by univariable linear regression to generate unadjusted effect estimates. Variables with *p*-values ≤ 0.25 in the univariable analyses and variables thought to be of clinical importance independent of their p-values were added in the multivariable models. Variables without significant effects in multivariable regression were excluded from the final models, with the exception of age, gender, disease duration, and anti-NR2 antibodies, regarded as important regardless of their significance levels. In the final models for memory test scores, also education levels and hippocampal volumes were included. A significance level of $p < 0.05$ was used.

3.7.2 Paper IV:

Global GM and WM volumes, GM volume/TIV and WM volume/TIV of 60 pSS patients and 60 healthy control subjects were compared using paired samples *t*-tests.

Models for GM and WM volumes in the pSS patients were established by testing potential explanatory variables with univariable linear regression to generate unadjusted effect estimates. As in Paper II, we first included all variables with $p \leq 0.25$ and variables thought to be of importance independent of *p*-values, and later excluded all variables without significance from the final multivariable models, with the exceptions of age, gender and disease duration. A significance threshold of $p < 0.05$ was used.

4. Summary of results:

4.1 Paper I

In this review article, some of the proposed mechanisms for NPSLE were discussed, with a main focus on the role of anti-NR2 antibodies. The murine experiments as well as the known role of the NMDAR for memory formation provide substantial support that anti-NR2 antibodies influence cognitive function. The results of the human SLE studies are conflicting: in some studies anti-NR2 antibodies are reported to have an effect on cognitive function, mood, and to be associated with other NPSLE manifestations, while no such associations are evident in other studies.

4.2 Paper II

4.2.1 Anti-NR2 antibodies

Six patients had anti-NR2 antibodies above cut-off in CSF, five out of them had increased IgG index, indicating intrathecal IgG production. The one without increased IgG index also had the lowest anti-NR2 antibody concentration out of the six patients. Another patient had increased IgG index without anti-NR2 antibodies above cut-off neither in serum nor in CSF. Three pSS patients had increased Q-albumin indicating leakage over the BBB; one of these patients had anti-NR2 antibodies above cut-off in serum (ratio 1.26), while none of them had anti-NR2 antibodies above cut-off in CSF.

4.2.2 Hippocampal size

We found that patients with pSS had smaller hippocampi than age- and gender matched healthy control subjects (mean 8.15 cm^3 ± SD 0.98 cm^3 versus 8.49 cm^3 ± 0.88 cm^3, p = 0.01).

Patients with anti-NR2 antibodies in CSF had smaller hippocampi than those without these antibodies (7.43 ± 0.71 cm^3 and 8.37 cm^3 ± 0.92 cm^3, respectively; p = 0.02). However, patients with anti-NR2 antibodies were older (67.4 versus 54.9 years, *p* =

0.01) and when correcting for age and gender, anti-NR2 antibodies lost the statistical significant influence ($p = 0.23$).

There were no associations between hippocampal size and aPL, anti-SSA antibodies, disease duration or present use of prednisolone.

There were no differences in hippocampal sizes between patients with and without mental depression.

4.2.3 Cognition

The pSS patients as a group had memory test mean scores within 1 SD of expected (based on the norms of the tests), however; for all tests there were some patients with impairment.

We found decreasing test performance in six of ten memory tests (WMS-R visual memory, WMS-R delayed memory, TPT dominant hand time, TPT both hands time, TPT total time and TPT memory) with increasing levels of anti-NR2 antibodies in serum. Further, there were decreasing test performance in eight of the ten tests (WMS-R verbal memory, WMS-R general memory, WMS-R delayed memory, TPT dominant hand time, TPT both hands time, TPT total time, TPT memory and TPT location) with increasing level of anti-NR2 antibodies in CSF.

Patients with aPL had lower test scores in three of the ten memory tests (WMS-R verbal memory, WMS-R general memory and TPT both hands time). Use of antimalarials had a significant or close to significant positive effect on performance in the WMS-R tests in univariable analyses, however, no effect was found in multivariable analyses.

There were no differences in memory performance between patients with or without mental depression.

There were no associations between hippocampal volumes and performance of any memory tests when anti-NR2 antibodies in CSF were included in the multivariable models. However, when instead anti-NR2 antibodies in serum were included,

decreasing hippocampal volumes were associated with impaired performance in all WMS-R tests except WMS-R verbal.

4.2.4 Mental depression

The patients had higher BDI scores than the healthy subjects (median 9, range 0-38 versus 2, 0-16), $p = 0.001$.

A higher proportion of the patients with mental depression had anti-NR2 antibodies in serum above cut-off compared with the non-depressed patients (35% vs 13%, $p = 0.04$), while no differences were revealed regarding anti-NR2 antibodies in CSF. There were no differences in memory test scores between patients with or without mental depression.

4.3 Paper III

There were no brain regions where GM volumes differed between SLE and pSS patients, and there were no interaction between disease groups and anti-NR2 antibodies. The SLE- and pSS patients with CSF anti-NR2 antibodies displayed lower GM volume in the hippocampi compared to the patients without CSF anti-NR2 antibodies. No effects from disease duration, aPL, or present use of corticosteroids were evident on hippocampal volumes, and there were no interactions between anti-NR2 antibodies and the other explanatory covariates. Finally, anti-NR2 antibodies had no influence on brain volumes in other regions than the hippocampus/para-hippocampus.

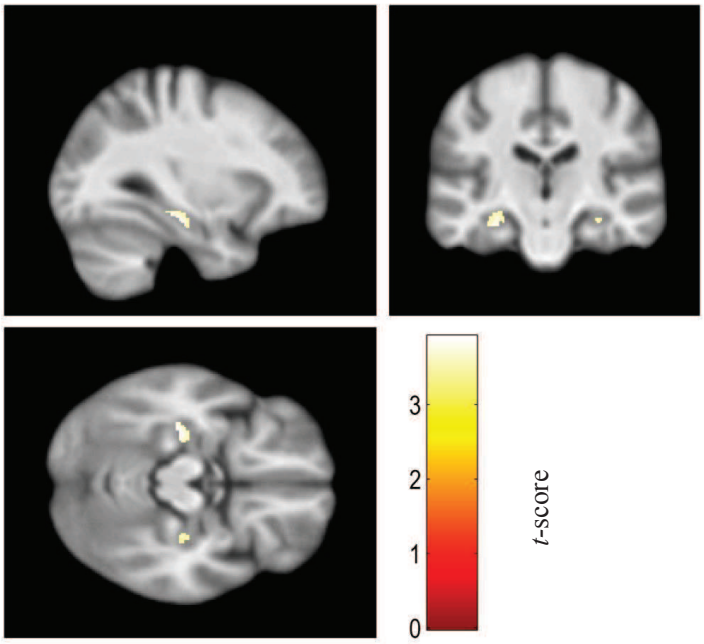

Figure 6: Clusters of voxels (yellow) within the hippocampus where patients with anti-NR2 antibodies in CSF had less GM compared to patients without anti-NR2 antibodies in CSF overlaid on mean image of all patients ($p < 0.05$, FWE corrected). The cluster colour represents the statistical significance of GM decrease.

4.4 Paper IV

The pSS patients had less cerebral WM compared to the age-and gender matched healthy subjects, 540 cm^3 (SD 63 cm^3) and 564 cm^3 (SD 56 cm^3), respectively, p=0.02. The WM proportion of the TIV were slightly lower in the pSS patients compared to the healthy subjects 39.6%, (95% confidence interval [CI] 39.1%, 40.0%) versus 40.1% (CI 39.7%, 40.6%), p = 0.03. In voxel-wise analyses, there were no localized WM atrophies in the pSS patients. There were no global or localized GM atrophy in the pSS patients. Mean GM volume were 546 cm^3 (SD 46 cm^3) in the pSS patients and 555 cm^3 (SD 51 cm^3) in the healthy control subjects, p = 0.21.

In the pSS patients, only gender had a significant effect on GM and WM volumes, and we could not reveal any effect from age, anti-SSA/SSB antibodies, aPL, WMH, mental depression, headache, peripheral vascular disorders (subclavian stenosis, stenosis or aneurysm of the carotid arteries, aorta sclerosis, operated aortic coarctation), hypertension or number of AECG criteria fulfilled (presumed to represent a surrogate measure of disease severity).

5. Discussion

Cerebral manifestations are common in both SLE and pSS patients, and it has been our hypothesis that some of the pathogenetic mechanisms may be shared in these diseases. Strong evidence point to a role for anti-NR2 antibodies in NPSLE; and the results in Papers II and III indicate that they may also be operative in pSS. We discovered an association between anti-NR2 antibodies and hippocampal atrophy in both SLE and pSS patients, in line with the animal models. We explored whether pSS patients had evidence of cerebral atrophy compared to age- and gender matched healthy control subjects, and finally if disease factors that have been associated with cerebral atrophy in SLE also may influence the brain in pSS. We found that global WM volumes were lower in pSS patients compared to healthy control subjects, while no GM atrophies were demonstrated, and none of the tested disease associated factors had any influence on WM- or GM volumes.

5.1 Evaluation of main findings

5.1.1 Paper I

Experimental conditions in animal studies are tightly controlled; the mice are of same genetic strain, are in the same environment and are exposed (or not) to identical antibodies in a controlled dose. In humans all these factors vary between the individuals, and several possible pathogenetic factors (e.g. other autoantibodies) may coexist. These biological variations as well as methodological differences probably accounts for at least some of the diversity in the results of the human studies.

5.1.1.1 Evaluation of cognitive function

Cognitive function was evaluated in 11 of the 13 reviewed studies. Structured neuropsychological testing was performed in all patients in six studies (114, 115, 118, 123-125). In one study neuropsychological assessment was carried out only when cognitive impairment was suspected (164), and in three studies cognitive function was clinically assessed by rheumatologists, neurologists or psychologists, at study

inclusion (78, 119) or later by medical record review (121). These different approaches to neuropsychological testing, the selection of neuropsychological tests applied, the norms for the test scores, and cut-off for abnormal test scores obviously influence the reported prevalences for cognitive dysfunction in the different study cohorts.

5.1.1.2 Anti-NR2 antibody assays

There are no internationally accepted standards for performing or reporting anti-NR2 antibody assays in serum or in CSF. In the published papers, there are apparent differences in the methods such as use of plates, different antigen peptide sequences to which the anti-NR2 antibodies bind, use of different concentrations of the peptide, and different enzyme-linked detection antibodies. Finally, different cut-off values for anti-NR2 antibodies are used, exemplified by mean OD for healthy control subjects + 2 SD (116), + 3 SD (117), or twice the highest level measured in healthy control subjects (123).

5.1.1.3 Serum versus CSF

Clinical effects seem to be more consistently reported when anti-NR2 antibodies are measured in CSF (three out of three studies) than in serum (three out of ten studies). Also, no cerebral effects seem to occur unless an opening of the BBB is induced. The reason why anti-NR2 antibodies in serum give rise to cerebral involvement could thus possibly be due to the passing of these antibodies through the BBB and into the CSF.

5.1.1.4 Methods for assessing potential effects of anti-NR2 antibodies on cognitive function

In most of the published studies an approach with dichotomizing subjects into groups with- or without cognitive impairment, and into anti-NR2 antibody positive or negative, has been applied. A more sensitive approach could be to use raw scores of neuropsychological tests as well as actual OD from the ELISA test, and the only study using this method, is also the only study where an association between anti-NR2 antibodies and cognitive impairment have been demonstrated (123).

5.1.1.5 Study design

The optimal design for studying the clinical effects of anti-NR2 autoantibodies would be a large longitudinal study in which anti-NR2 antibodies, as well as other relevant autoantibodies and biomarkers are measured repeatedly, and all neuropsychiatric events registered over time. However, temporary changes in anti-NR2 antibodies may possibly occur in the intervals between the examinations, and "training effects" can result from repeated neuropsychological assessments.

Two studies reviewed in Paper I had a longitudinal design. In one of these, anti-NR2 antibodies were measured in CSF in patients hospitalized for active NPSLE manifestations, and six months later. At follow-up the NPSLE manifestations had resolved and anti-NR2 antibody concentrations were lower in all but one patient (122). In the other longitudinal study, serum anti-NR2 antibodies were measured and cognitive function tested at inclusion and after 5 years (117). In that study, no associations between anti-NR2 antibodies and cognitive function were found.

5.1.2 Paper II

5.1.2.1 Hippocampal size

It is known that patients with mental depression have smaller hippocampi than healthy subjects (165). We could not confirm such an effect on hippocampal sizes when we performed regression analyses, neither when mental depression was added as a binary variable (present or not), nor when the actual BDI scores were used.

In one study of SLE patients with high disease activity and a high prevalence of aPL, hippocampal atrophy was associated with presence of aPL, disease duration, and cumulative corticosteroid dose (150). Only eight of our patients had aPL in serum and there were no differences in the hippocampal sizes between patients with and without aPL. Neither did disease duration influence hippocampal size in our pSS patients. This could reflect differences in the two disease entities. Another explanation could be that our pSS cohort is population-based and thus characterized by lower disease activity. This could imply less detrimental effect on the hippocampi over time. More studies are therefore needed to clarify these matters. Finally, no associations between use of

corticosteroids and hippocampal size were revealed in our study. Applying cumulative dose of corticosteroids might have given another result, but it was not possible to obtain these data from our cohort.

5.1.2.2 Cognition

The associations between CSF anti-NR2 antibodies and memory test performances that we observed are in accordance with the known function of the NMDA receptors and confirm experimental animal studies. We also revealed an effect of anti-NR2 antibodies in serum in six of the memory tests. This is in accordance with the findings in a study of SLE patients and indicates that the BBB not necessarily needs to be opened (123).

Memory test performance was not influenced by hippocampal size when anti-NR2 antibodies in CSF were included in the multivariable regression analyses. A possible explanation could be that memory impairment is a result of disturbed neuronal function rather than neuronal death observed in MRI as smaller hippocampi. However, memory performances were mostly within normal range, and statistics could be underpowered to detect minor effects. Also limitations in the precision of the hippocampus size estimation could reduce our ability to reveal subtle effects.

5.1.2.3 Mental depression

In accordance with previous observations anti-NR2 antibodies in serum above cut-off were more frequent in pSS patients with mental depression than in pSS patients without (109, 114, 123). No effect of anti-NR2 antibodies in CSF on mental depression were revealed, this lack of association could be due to limited statistical power with only two pSS patients with CSF anti-NR2 antibodies and mental depression in our cohort.

5.1.3 Paper III

The SLE- and pSS patients with anti-NR2 antibodies had loss of GM in the hippocampi in line with animal studies, while anti-NR2 antibodies were not associated with changes of GM in other brain areas. The most likely explanation for the localized GM loss is neuronal excitoxicity taking place in the hippocampi in which the NR2b

receptors appear at particular high densities. More subtle effects on GM may have existed in other brain areas than we were able to reveal, due to the limited statistical power (only 14 of the patients in the combined cohort had anti-NR2 antibodies in CSF).

5.1.4 Paper IV

5.1.4.1 Global GM and WM volumes

We observed less WM in the pSS patients than in the healthy control subjects, while there were no statistical differences regarding GM. However, we cannot conclude that cerebral WM is more vulnerable to disease associated factors than GM, as the study was statistically underpowered to detect minor differences in GM. The clinical significance and implications of the findings might have increased if data on cognitive function had been applied together with volumetric data. Analyses of cognitive results were unfortunately not completed and data thus not available when the analyses for Paper IV were performed.

The smaller TIV in the patient group probably represent selection bias of "super-normal" healthy control subjects. The MRI scannings were performed interleaved and data quality was the same across the groups as judged by visual inspection of the images and evaluation of the covariance matrices from the preprocessing. These factors limit the risk of systematic differences in image acquisition or data quality. An alternative explanation could be that the pSS patients had premorbid smaller sculls than the general population.

5.1.4.2 Voxel-wise analyses

The lack of localized differences in GM or WM volumes between the pSS patients and the control subjects contrasts another VBM study in which widespread atrophy of both GM and WM was reported in the patients (149). An older version of the SPM software was applied in that study and the authors also applied a lenient correction for multiple comparison, FDR-correction with $p < 0.05$. This correction has turned out to be inappropriate in the VBM context and can no longer be selected in updated versions of SPM (166). The FWE-correction, on the other hand, may be too harsh in a whole-brain

setting, but we did not disclose any significant voxels at an uncorrected $p < 0.001$ level either. Also in this setting we may have been statistically underpowered to detect more subtle differences between the groups.

5.1.4.3 Multivariable regression analyses

We could not reveal disease specific factors that influenced WM in the multivariable analyses. A bit surprisingly only gender, and not age, turned out to have statistical effect on GM and WM volumes, although the age range in our cohort was almost 60 years. By plotting WM volumes against age and adding a quadratic instead of linear fit line, it became evident that the association was non-linear with increasing WM volumes up to approximately 50 years, followed by a decline thereafter. By adding squared age to the multivariable regression analyses, WM increased with increasing age (β 7.8, adjusted R^2 0.24, $p = 0.01$), but at higher ages, WM volumes decreased with increasing age (squared age β -0.07, adjusted R^2 0.24, $p = 0.02$). These findings are in line with a large study exploring the effect of age on GM and WM volumes in healthy subjects (167). No effects of squared age on GM volumes, CSF volumes or TIV were revealed by our study.

Regarding other clinical variables and disease related factors that we tested, it is a possibility that we may have been statistically underpowered to reveal any effects.

5.2 Evaluation of methods

5.2.1 Recruitment of patients and healthy subjects

We aimed to include all pSS and SLE patients in our region to obtain a close-to population based cohort for study. To minimalize the variance caused by methodological differences participants were examined by the same specialists, and within the same MRI machine and with the same scanning protocol. Most patients accepted the extensive research protocol including cerebral MRI and lumbar puncture thus giving us a unique possibility to explore associations between CSF variables (e.g. autoantibodies and cytokines), MRI findings and cognitive function. Though there are advantages with a single-centre cohort, it would have been advantageous to include

even more patients, especially when examining the potential effects of auto-antibodies with low prevalence, such as anti-NR2 antibodies in CSF (present in 8 of the SLE- and 6 of the pSS patient) and aPL in blood (present in 8 of the pSS patients).

Inclusion of some control subjects from the hospital staff may have caused a bias towards subjects with higher education than the general population. The larger TIV in the healthy control subjects compared with the pSS patients could be an indication of that. Lack of education level data in the control subjects prevented us from comparing education levels between the groups.

5.2.2 Anti-NR2 antibody analyses

It has been claimed that anti-NR2 antibodies must be present above a certain threshold concentration to cause neuronal apoptosis, while at lower concentrations they lead to neuronal dysfunction only (112, 168). Whether there is an even lower level below which no detectable influences on neurons are evident, is not known. If such a level existed, this would have been the basis for a "biological cut-off" for the anti-NR2 assays. We applied an "analytical" cut-off for anti-NR2 antibodies in serum and in CSF approximately 3SD above the mean concentration for all control subjects.

5.2.3 MRI analyses

Computer-based analyses are less operator dependent and more quickly performed compared with visual assessment or manual measures. However, differences in settings can cause considerable variations in the results. We applied default settings in preprocessing advised by the software developers (http://dbm.neuro.uni-jena.de/vbm/).

We found that WM and GM volumes were approximately of identical size in the pSS patients and healthy subjects. This contrasts previous literature in which GM volumes usually exceed WM volumes (169, 170). There are some possible explanations: In a previous study it was demonstrated that with increasing age GM volumes declined while WM volumes increased up to around age 45 years, and thereafter declined. The age of the cohort studied will thus be of major importance (167). Another explanation could be that suboptimal settings in the MRI-protocol caused reduced WM/GM

contrast resulting in GM being misinterpreted as WM, though not to such an extent that it became evident when the segmentation was visually checked.

6. Conclusion

Anti-NR2 antibodies represent one of several pathogenetic factors that can lead to cognitive dysfunction and mental depression in patients with SLE. We found that these antibodies are operative in patients with pSS also. Thus, anti-NR2 antibodies may represent a generic mechanism for cognitive dysfunction and mental depression in autoimmune diseases. Also, aPL were associated with reduced memory test scores. pSS patients had smaller hippocampi than healthy subjects as previously reported in SLE patients.

SLE and pSS patients with anti-NR2 antibodies in CSF had less hippocampal GM compared to patients without these antibodies in CSF. This indicates that anti-NR2 antibodies can cause neuronal death in humans revealed as hippocampal atrophy, as previously reported in animal studies.

Finally, pSS patients had a diffuse WM loss compared to healthy control subjects, suggesting a detrimental effect of disease-related factors on WM. We found no differences in GM volumes between the pSS patients and the healthy control subjects. We cannot exclude that this was due to limited statistical power, and therefore inability to reveal small differences.

7. Future perspectives

Our study was the first to discover anti-NR2 antibodies in patients with pSS, and to explore their effect on cognitive function, mental depression and hippocampal sizes. These results need to be confirmed in other studies in which a wider spectrum of memory tests is applied. This could possibly help to elucidate whether more specific memory functions, i.e. learning, immediate and delayed recall, and specific anatomical areas in which memory is being processed, are affected.

Also, the influence of aPL on cognitive function in pSS patients needs to be confirmed, and anti-P must be further explored both in SLE and pSS patients.

New generations of MRI machines with higher resolution and improved scanning protocols can more precisely identify and measure regions of interest in the hippocampi and other brain areas. MRI diffusion tensor imaging is a better approach than VBM to reveal structural changes in WM, and should be another modality to use in future studies.

8. Acknowledgements

"Mum, you're working with typing letters" my four-year old daughter told me. Letters were words, words have been sentences and sentences have become papers and the papers have been the basis for thesis.

I would like to thank Roald Omdal, I am so grateful for having you as my supervisor. You are a great example with your extensive knowledge, enthusiasm, integrity and the way you care for people around you. I have always felt welcome with small and large questions, you have unique skills in seeing solutions, and I have always been encouraged in discussions with you.

I would also thank Erna Harboe for recruiting me to the group and Lasse Gøransson for making me believe in starting a PhD work, and both for the great work you have done in recruiting and examining the patients and for valuable feedback in the writing process.

Mona Beyer, your neuroimaging knowledge has been invaluable. Thank you for all support, from introducing me to the software, help in modelling and interpretation of the results. I still miss you in Stavanger.

Jan Terje Kvaløy, you have made the mysteries of statistics less mysterious. You have unique pedagogic skills, more than once have I started discussions with you, not even sure of what my questions were and you have been able to sort it out. Thank you for your help, patience and encouragement.

Thank you Stian Maroni for your enthusiastic introduction to neuropsychology, for the great work you have done in examining all the patients and help in test selection and interpretation.

I would also thank Simone Appenzeller, Ole Jacob Greve and Ingeborg Kvivik for all help in acquisition, analyses and interpretation of data and for valuable feedback in writing and Tone Berit Heng for performing the anti-NR2 antibody analyses of serum.

It has been of great value to be part of the clinical immunology research group. The members of this group have various professions; the different point of views have broadened my perspective. Also socially it has been great to be part of this group. I would especially like to thank Katrine Brække Norheim, Svein Joar Johnsen and Anne Bolette Tjensvoll; I have had so much fun with you during these years, but you have also been important support in demanding periods. I would also thank the other members; Tore Grimstad, Kjetil Bårdsen, Grete Jonsson, Siri Lunde, Oddbjørn Bjordal, Cato Brede, Eivind Larssen, Elin-Johanne Katle, Inger Marie Skoie and Mari Mæland Nilsen.

I would like to thank:

- Stein Tore Nilsen, Margot Viste, Fredrik Feyling, Torbjørn Aarsland and all other colleagues at *Forskningens hus*.

- The librarians at SUS

- Western Norway Regional Health Authorities for funding my work

- Contributors on the SPM mailing list for all support in the use of SPM

- All patients and healthy control subjects who participated in these studies. Your participation did this work possible.

Finally I would like to thank my friends and family. My parents Åslaug and Jens, and Elin and Håvard for all love and support, Torborg and Bjørn and all brothers- and sisters in law, I am happy to have such a great family. Last, but not least, I would like to thank my everyday joy, Liv Sigrid, Sunniva, Miriam and Leiv Halvor, you are the best in my life. I love you.

9. References

1. Medzhitov R, Janeway C, Jr. Innate immunity. N Engl J Med. 2000;343(5):338-44.
2. Dempsey PW, Vaidya SA, Cheng G. The art of war: Innate and adaptive immune responses. Cellular and molecular life sciences : CMLS. 2003;60(12):2604-21.
3. Moser M, Leo O. Key concepts in immunology. Vaccine. 2010;28 Suppl 3:C2-13.
4. Delves PJ, Roitt IM. The immune system. First of two parts. N Engl J Med. 2000;343(1):37-49.
5. Delves PJ, Roitt IM. The immune system. Second of two parts. N Engl J Med. 2000;343(2):108-17.
6. Wahren-Herlenius M, Dorner T. Immunopathogenic mechanisms of systemic autoimmune disease. Lancet. 2013;382(9894):819-31.
7. Vignali DAA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol. 2008;8(7):523-32.
8. Grossman WJ, Verbsky JW, Barchet W, Colonna M, Atkinson JP, Ley TJ. Human T regulatory cells can use the perforin pathway to cause autologous target cell death. Immunity. 2004;21(4):589-601.
9. Cooper GS, Bynum ML, Somers EC. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. J Autoimmun. 2009;33(3-4):197-207.
10. Graham RR, Ortmann WA, Langefeld CD, Jawaheer D, Selby SA, Rodine PR, et al. Visualizing human leukocyte antigen class II risk haplotypes in human systemic lupus erythematosus. Am J Hum Genet. 2002;71(3):543-53.
11. Gateva V, Sandling JK, Hom G, Taylor KE, Chung SA, Sun X, et al. A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. Nat Genet. 2009;41(11):1228-33.
12. Truedsson L, Bengtsson AA, Sturfelt G. Complement deficiencies and systemic lupus erythematosus. Autoimmunity. 2007;40(8):560-6.
13. Feero WG, Guttmacher AE, Collins FS. Genomic medicine--an updated primer. N Engl J Med. 2010;362(21):2001-11.
14. Lessard CJ, Li H, Adrianto I, Ice JA, Rasmussen A, Grundahl KM, et al. Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjogren's syndrome. Nat Genet. 2013;45(11):1284-92.
15. Hom G, Graham RR, Modrek B, Taylor KE, Ortmann W, Garnier S, et al. Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. N Engl J Med. 2008;358(9):900-9.
16. Deapen D, Escalante A, Weinrib L, Horwitz D, Bachman B, Roy-Burman P, et al. A revised estimate of twin concordance in systemic lupus erythematosus. Arthritis Rheum. 1992;35(3):311-8.
17. Meda F, Folci M, Baccarelli A, Selmi C. The epigenetics of autoimmunity. Cellular & molecular immunology. 2011;8(3):226-36.

18. Cho JH, Gregersen PK. Genomics and the multifactorial nature of human autoimmune disease. N Engl J Med. 2011;365(17):1612-23.
19. Miao CG, Yang YY, He X, Huang C, Huang Y, Zhang L, et al. The emerging role of microRNAs in the pathogenesis of systemic lupus erythematosus. Cellular signalling. 2013;25(9):1828-36.
20. Lleo A, Battezzati PM, Selmi C, Gershwin ME, Podda M. Is autoimmunity a matter of sex? Autoimmun Rev. 2008;7(8):626-30.
21. Selmi C, Brunetta E, Raimondo MG, Meroni PL. The X chromosome and the sex ratio of autoimmunity. Autoimmun Rev. 2012;11(6-7):A531-7.
22. Davidson A, Diamond B. Autoimmune diseases. N Engl J Med. 2001;345(5):340-50.
23. Edworthy S. Clinical manifestations of systemic lupus erythematosus. In: Harris ED, Budd RC, Firestein GS, Genovese MC, Sergent JS, Ruddy S, et al., editors. Kelley's textbook of rheumatology. 7th ed: Elsevier Saunders; 2004. p. 1204-15.
24. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40(9):1725.
25. Eilertsen GO, Becker-Merok A, Nossent JC. The influence of the 1997 updated classification criteria for systemic lupus erythematosus: epidemiology, disease presentation, and patient management. J Rheumatol. 2009;36(3):552-9.
26. 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. J Rheumatol. 2000;27(3):685-91.
27. 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 Res (Hoboken). 2014;66(11):1710-7.
28. 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.
29. 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.
30. Harboe E, Tjensvoll AB, Maroni S, Goransson LG, Greve OJ, Beyer MK, et al. Neuropsychiatric syndromes in patients with systemic lupus erythematosus and primary Sjogren syndrome: a comparative population-based study. Ann Rheum Dis. 2009;68(10):1541-6.
31. ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature. The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. Arthritis Rheum. 1999;42(4):599-608.
32. Jonsson R, Vogelsang P, Volchenkov R, Espinosa A, Wahren-Herlenius M, Appel S. The complexity of Sjogren's syndrome: novel aspects on pathogenesis. Immunol Lett. 2011;141(1):1-9.
33. Goransson LG, Haldorsen K, Brun JG, Harboe E, Jonsson MV, Skarstein K, et al. The point prevalence of clinically relevant primary Sjogren's syndrome in two Norwegian counties. Scand J Rheumatol. 2011;40(3):221-4.

34. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis. 2002;61(6):554-8.
35. Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H, et al. American College of Rheumatology classification criteria for Sjogren's syndrome: a data-driven, expert consensus approach in the Sjogren's International Collaborative Clinical Alliance cohort. Arthritis Care Res (Hoboken). 2012;64(4):475-87.
36. Soliotis FC, Mavragani CP, Moutsopoulos HM. Central nervous system involvement in Sjogren's syndrome. Ann Rheum Dis. 2004;63(6):616-20.
37. Brodal P. Sentralnervesystemet. 4. utg. ed. Oslo: Universitetsforl.; 2007.
38. Brown PD, Davies SL, Speake T, Millar ID. Molecular mechanisms of cerebrospinal fluid production. Neuroscience. 2004;129(4):957-70.
39. Ransohoff RM, Engelhardt B. The anatomical and cellular basis of immune surveillance in the central nervous system. Nat Rev Immunol. 2012;12(9):623-35.
40. Banks WA, Erickson MA. The blood-brain barrier and immune function and dysfunction. Neurobiol Dis. 2010;37(1):26-32.
41. Neuwelt E, Abbott NJ, Abrey L, Banks WA, Blakley B, Davis T, et al. Strategies to advance translational research into brain barriers. Lancet neurology. 2008;7(1):84-96.
42. Engelhardt B, Coisne C. Fluids and barriers of the CNS establish immune privilege by confining immune surveillance to a two-walled castle moat surrounding the CNS castle. Fluids and barriers of the CNS. 2011;8(1):4.
43. Knopf PM, Harling-Berg CJ, Cserr HF, Basu D, Sirulnick EJ, Nolan SC, et al. Antigen-dependent intrathecal antibody synthesis in the normal rat brain: tissue entry and local retention of antigen-specific B cells. J Immunol. 1998;161(2):692-701.
44. Winfield JB, Shaw M, Silverman LM, Eisenberg RA, Wilson HA, 3rd, Koffler D. Intrathecal IgG synthesis and blood-brain barrier impairment in patients with systemic lupus erythematosus and central nervous system dysfunction. Am J Med. 1983;74(5):837-44.
45. Abbott NJ, Mendonca LL, Dolman DE. The blood-brain barrier in systemic lupus erythematosus. Lupus. 2003;12(12):908-15.
46. Hossain M, Sathe T, Fazio V, Mazzone P, Weksler B, Janigro D, et al. Tobacco smoke: a critical etiological factor for vascular impairment at the blood-brain barrier. Brain Res. 2009;1287:192-205.
47. Qi X, Inagaki K, Sobel RA, Mochly-Rosen D. Sustained pharmacological inhibition of deltaPKC protects against hypertensive encephalopathy through prevention of blood-brain barrier breakdown in rats. J Clin Invest. 2008;118(1):173-82.
48. Catani M, Dell'acqua F, Bizzi A, Forkel SJ, Williams SC, Simmons A, et al. Beyond cortical localization in clinico-anatomical correlation. Cortex; a journal devoted to the study of the nervous system and behavior. 2012;48(10):1262-87.
49. ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature. Appendix A: Case Definitions for Neuropsychiatric Syndromes in Systemic Lupus Erythematosus Arthritis and Rheumatism. 1999;41(4).

50. Carbotte RM, Denburg SD, Denburg JA. Prevalence of cognitive impairment in systemic lupus erythematosus. J Nerv Ment Dis. 1986;174(6):357-64.
51. Waterloo K, Omdal R, Husby G, Mellgren SI. Neuropsychological function in systemic lupus erythematosus: a five-year longitudinal study. Rheumatology (Oxford). 2002;41(4):411-5.
52. Hanly JG, Cassell K, Fisk JD. Cognitive function in systemic lupus erythematosus: results of a 5-year prospective study. Arthritis Rheum. 1997;40(8):1542-3.
53. Carlomagno S, Migliaresi S, Ambrosone L, Sannino M, Sanges G, Di Iorio G. Cognitive impairment in systemic lupus erythematosus: a follow-up study. J Neurol. 2000;247(4):273-9.
54. Segal BM, Mueller BA, Zhu X, Prosser R, Pogatchnik B, Holker E, et al. Disruption of brain white matter microstructure in primary Sjogren's syndrome: evidence from diffusion tensor imaging. Rheumatology (Oxford). 2010;49(8):1530-9.
55. Mataro M, Escudero D, Ariza M, Sanchez-Ojanguren J, Latorre P, Junque C, et al. Magnetic resonance abnormalities associated with cognitive dysfunction in primary Sjogren syndrome. J Neurol. 2003;250(9):1070-6.
56. Lafitte C, Amoura Z, Cacoub P, Pradat-Diehl P, Picq C, Salachas F, et al. Neurological complications of primary Sjogren's syndrome. J Neurol. 2001;248(7):577-84.
57. Le Guern V, Belin C, Henegar C, Moroni C, Maillet D, Lacau C, et al. Cognitive function and 99mTc-ECD brain SPECT are significantly correlated in patients with primary Sjogren syndrome: a case-control study. Ann Rheum Dis. 2010;69(1):132-7.
58. Martinez S, Caceres C, Mataro M, Escudero D, Latorre P, Davalos A. Is there progressive cognitive dysfunction in Sjogren Syndrome? A preliminary study. Acta Neurol Scand. 2010;122(3):182-8.
59. Johnson RT, Richardson EP. The neurological manifestations of systemic lupus erythematosus. Medicine (Baltimore). 1968;47(4):337-69.
60. Devinsky O, Petito CK, Alonso DR. Clinical and neuropathological findings in systemic lupus erythematosus: the role of vasculitis, heart emboli, and thrombotic thrombocytopenic purpura. Ann Neurol. 1988;23(4):380-4.
61. Ellis SG, Verity MA. Central nervous system involvement in systemic lupus erythematosus: a review of neuropathologic findings in 57 cases, 1955--1977. Semin Arthritis Rheum. 1979;8(3):212-21.
62. Hanly JG, Walsh NM, Sangalang V. Brain pathology in systemic lupus erythematosus. J Rheumatol. 1992;19(5):732-41.
63. de la Monte SM, Hutchins GM, Gupta PK. Polymorphous meningitis with atypical mononuclear cells in Sjogren's syndrome. Ann Neurol. 1983;14(4):455-61.
64. Gerraty RP, McKelvie PA, Byrne E. Aseptic meningoencephalitis in primary Sjogren's syndrome. Response to plasmapheresis and absence of CNS vasculitis at autopsy. Acta Neurol Scand. 1993;88(4):309-11.
65. Yaguchi H, Houzen H, Kikuchi K, Hata D, Ura S, Takeda T, et al. An autopsy case of Sjogren's syndrome with acute encephalomyelopathy. Internal medicine (Tokyo, Japan). 2008;47(19):1675-80.

66. Delalande S, de Seze J, Fauchais AL, Hachulla E, Stojkovic T, Ferriby D, et al. Neurologic manifestations in primary Sjogren syndrome: a study of 82 patients. Medicine (Baltimore). 2004;83(5):280-91.
67. Trysberg E, Nylen K, Rosengren LE, Tarkowski A. Neuronal and astrocytic damage in systemic lupus erythematosus patients with central nervous system involvement. Arthritis Rheum. 2003;48(10):2881-7.
68. Govoni M, Bajocchi G, Rizzo N, Tola MR, Caniatti L, Tugnoli V, et al. Neurological involvement in primary Sjogren's syndrome: clinical and instrumental evaluation in a cohort of Italian patients. Clin Rheumatol. 1999;18(4):299-303.
69. Escudero D, Latorre P, Codina M, Coll-Canti J, Coll J. Central nervous system disease in Sjogren's syndrome. Ann Med Interne (Paris). 1995;146(4):239-42.
70. Harris EN, Gharavi AE, Boey ML, Patel BM, Mackworth-Young CG, Loizou S, et al. Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet. 1983;2(8361):1211-4.
71. Futrell N, Millikan C. Frequency, etiology, and prevention of stroke in patients with systemic lupus erythematosus. Stroke. 1989;20(5):583-91.
72. Chiang CH, Liu CJ, Chen PJ, Huang CC, Hsu CY, Chan WL, et al. Primary Sjogren's syndrome and risk of ischemic stroke: a nationwide study. Clin Rheumatol. 2014;33(7):931-7.
73. Haga HJ, Jacobsen EM, Peen E. Incidence of thromboembolic events in patients with primary Sjogren's syndrome. Scand J Rheumatol. 2008;37(2):127-9.
74. Cervera R, Garcia-Carrasco M, Font J, Ramos M, Reverter JC, Munoz FJ, et al. Antiphospholipid antibodies in primary Sjogren's syndrome: prevalence and clinical significance in a series of 80 patients. Clin Exp Rheumatol. 1997;15(4):361-5.
75. Hanly JG, Hong C, Smith S, Fisk JD. A prospective analysis of cognitive function and anticardiolipin antibodies in systemic lupus erythematosus. Arthritis Rheum. 1999;42(4):728-34.
76. Menon S, Jameson-Shortall E, Newman SP, Hall-Craggs MR, Chinn R, Isenberg DA. A longitudinal study of anticardiolipin antibody levels and cognitive functioning in systemic lupus erythematosus. Arthritis Rheum. 1999;42(4):735-41.
77. Isshi K, Hirohata S. Association of anti-ribosomal P protein antibodies with neuropsychiatric systemic lupus erythematosus. Arthritis Rheum. 1996;39(9):1483-90.
78. Hanly JG, Urowitz MB, Siannis F, Farewell V, Gordon C, Bae SC, et al. Autoantibodies and neuropsychiatric events at the time of systemic lupus erythematosus diagnosis: results from an international inception cohort study. Arthritis Rheum. 2008;58(3):843-53.
79. Karassa FB, Afeltra A, Ambrozic A, Chang DM, De Keyser F, Doria A, et al. Accuracy of anti-ribosomal P protein antibody testing for the diagnosis of neuropsychiatric systemic lupus erythematosus: an international meta-analysis. Arthritis Rheum. 2006;54(1):312-24.
80. Yoshio T, Hirata D, Onda K, Nara H, Minota S. Antiribosomal P protein antibodies in cerebrospinal fluid are associated with neuropsychiatric systemic lupus erythematosus. J Rheumatol. 2005;32(1):34-9.
81. Hirohata S, Arinuma Y, Takayama M, Yoshio T. Association of cerebrospinal fluid anti-ribosomal p protein antibodies with diffuse psychiatric/neuropsychological syndromes in systemic lupus erythematosus. Arthritis Res Ther. 2007;9(3):R44.

82. Matus S, Burgos PV, Bravo-Zehnder M, Kraft R, Porras OH, Farias P, et al. Antiribosomal-P autoantibodies from psychiatric lupus target a novel neuronal surface protein causing calcium influx and apoptosis. J Exp Med. 2007;204(13):3221-34.
83. Bravo-Zehnder M, Toledo EM, Segovia-Miranda F, Serrano FG, Benito MJ, Metz C, et al. Anti-ribosomal p protein autoantibodies from patients with neuropsychiatric lupus impair memory in mice. Arthritis & rheumatology (Hoboken, NJ). 2015;67(1):204-14.
84. Ersvaer E, Bertelsen LT, Espenes LC, Bredholt T, Boe SO, Iversen BM, et al. Characterization of ribosomal P autoantibodies in relation to cell destruction and autoimmune disease. Scand J Immunol. 2004;60(1-2):189-98.
85. Spezialetti R, Bluestein HG, Peter JB, Alexander EL. Neuropsychiatric disease in Sjogren's syndrome: anti-ribosomal P and anti-neuronal antibodies. Am J Med. 1993;95(2):153-60.
86. Bresnihan B, Oliver M, Williams B, Hughes GR. An antineuronal antibody cross-reacting with erythrocytes and lymphocytes in systemic lupus erythematosus. Arthritis Rheum. 1979;22(4):313-20.
87. West SG, Emlen W, Wener MH, Kotzin BL. Neuropsychiatric lupus erythematosus: a 10-year prospective study on the value of diagnostic tests. Am J Med. 1995;99(2):153-63.
88. Schulte-Pelkum J, Fritzler M, Mahler M. Latest update on the Ro/SS-A autoantibody system. Autoimmun Rev. 2009;8(7):632-7.
89. Hernandez-Molina G, Leal-Alegre G, Michel-Peregrina M. The meaning of anti-Ro and anti-La antibodies in primary Sjogren's syndrome. Autoimmun Rev. 2011;10(3):123-5.
90. Alexander EL, Ranzenbach MR, Kumar AJ, Kozachuk WE, Rosenbaum AE, Patronas N, et al. Anti-Ro(SS-A) autoantibodies in central nervous system disease associated with Sjogren's syndrome (CNS-SS): clinical, neuroimaging, and angiographic correlates. Neurology. 1994;44(5):899-908.
91. Hinson SR, Roemer SF, Lucchinetti CF, Fryer JP, Kryzer TJ, Chamberlain JL, et al. Aquaporin-4-binding autoantibodies in patients with neuromyelitis optica impair glutamate transport by down-regulating EAAT2. J Exp Med. 2008;205(11):2473-81.
92. Graber DJ, Levy M, Kerr D, Wade WF. Neuromyelitis optica pathogenesis and aquaporin 4. Journal of neuroinflammation. 2008;5:22.
93. Jarius S, Jacobi C, de Seze J, Zephir H, Paul F, Franciotta D, et al. Frequency and syndrome specificity of antibodies to aquaporin-4 in neurological patients with rheumatic disorders. Mult Scler. 2011;17(9):1067-73.
94. Moore PM. Neuropsychiatric systemic lupus erythematosus. In: G LR, editor. Systemic lupus erythematosus. 3rd ed. San Diego: Academic press; 1999. p. 575-8

95. Perez-Otano I, Schulteis CT, Contractor A, Lipton SA, Trimmer JS, Sucher NJ, et al. Assembly with the NR1 subunit is required for surface expression of NR3A-containing NMDA receptors. J Neurosci. 2001;21(4):1228-37.
96. Paoletti P, Bellone C, Zhou Q. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat Rev Neurosci. 2013;14(6):383-400.

97. Ozawa S, Kamiya H, Tsuzuki K. Glutamate receptors in the mammalian central nervous system. Prog Neurobiol. 1998;54(5):581-618.
98. Lauvsnes MB, Omdal R. Systemic lupus erythematosus, the brain, and anti-NR2 antibodies. J Neurol. 2012;259(4):622-9.
99. Mayer ML, Vyklicky L, Sernagor E. A physiologist's view of the N-methyl-D-Aspartate receptor: An allosteric ion channel with multiple regulatory sites Drug Development Research. 1989;17(4):263 - 80.
100. Sheng M, Pak DT. Ligand-gated ion channel interactions with cytoskeletal and signaling proteins. Annu Rev Physiol. 2000;62:755-78.
101. Martel MA, Wyllie DJ, Hardingham GE. In developing hippocampal neurons, NR2B-containing N-methyl-D-aspartate receptors (NMDARs) can mediate signaling to neuronal survival and synaptic potentiation, as well as neuronal death. Neuroscience. 2009;158(1):334-43.
102. Cooke SF, Bliss TV. Plasticity in the human central nervous system. Brain. 2006;129(Pt 7):1659-73.
103. Hardingham GE, Bading H. The Yin and Yang of NMDA receptor signalling. Trends Neurosci. 2003;26(2):81-9.
104. Hansen KB, Ogden KK, Yuan H, Traynelis SF. Distinct functional and pharmacological properties of Triheteromeric GluN1/GluN2A/GluN2B NMDA receptors. Neuron. 2014;81(5):1084-96.
105. Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med. 1994;330(9):613-22.
106. Gaynor B, Putterman C, Valadon P, Spatz L, Scharff MD, Diamond B. Peptide inhibition of glomerular deposition of an anti-DNA antibody. Proc Natl Acad Sci U S A. 1997;94(5):1955-60.
107. 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. Nat Med. 2001;7(11):1189-93.
108. Kowal C, DeGiorgio LA, Nakaoka T, Hetherington H, Huerta PT, Diamond B, et al. Cognition and immunity; antibody impairs memory. Immunity. 2004;21(2):179-88.
109. Huerta PT, Kowal C, DeGiorgio LA, Volpe BT, Diamond B. Immunity and behavior: antibodies alter emotion. Proc Natl Acad Sci U S A. 2006;103(3):678-83.
110. Kowal C, Degiorgio LA, Lee JY, Edgar MA, Huerta PT, Volpe BT, et al. Human lupus autoantibodies against NMDA receptors mediate cognitive impairment. Proc Natl Acad Sci U S A. 2006;103(52):19854-9.
111. Faust TW, Chang EH, Kowal C, Berlin R, Gazaryan IG, Bertini E, et al. Neurotoxic lupus autoantibodies alter brain function through two distinct mechanisms. Proc Natl Acad Sci U S A.107(43):18569-74.
112. Gono T, Takarada T, Fukumori R, Kawaguchi Y, Kaneko H, Hanaoka M, et al. NR2-reactive antibody decreases cell viability through augmentation of Ca(2+) influx in systemic lupus erythematosus. Arthritis Rheum. 2011;63(12):3952-9.
113. Yoshio T, Okamoto H, Hirohata S, Minota S. IgG anti-NR2 glutamate receptor autoantibodies from patients with systemic lupus erythematosus activate endothelial cells. Arthritis Rheum. 2013;65(2):457-63.

114. Lapteva L, Nowak M, Yarboro CH, Takada K, Roebuck-Spencer T, Weickert T, et al. Anti-N-methyl-D-aspartate receptor antibodies, cognitive dysfunction, and depression in systemic lupus erythematosus. Arthritis Rheum. 2006;54(8):2505-14.
115. Harrison MJ, Ravdin LD, Lockshin MD. Relationship between serum NR2a antibodies and cognitive dysfunction in systemic lupus erythematosus. Arthritis Rheum. 2006;54(8):2515-22.
116. Husebye ES, Sthoeger ZM, Dayan M, Zinger H, Elbirt D, Levite M, et al. Autoantibodies to a NR2A peptide of the glutamate/NMDA receptor in sera of patients with systemic lupus erythematosus. Ann Rheum Dis. 2005;64(8):1210-3.
117. Hanly JG, Robichaud J, Fisk JD. Anti-NR2 glutamate receptor antibodies and cognitive function in systemic lupus erythematosus. J Rheumatol. 2006;33(8):1553-8.
118. Gono T, Kawaguchi Y, Kaneko H, Nishimura K, Hanaoka M, Kataoka S, et al. Anti-NR2A antibody as a predictor for neuropsychiatric systemic lupus erythematosus. Rheumatology (Oxford). 2011;50(9):1578-85.
119. Yoshio T, Onda K, Nara H, Minota S. Association of IgG anti-NR2 glutamate receptor antibodies in cerebrospinal fluid with neuropsychiatric systemic lupus erythematosus. Arthritis Rheum. 2006;54(2):675-8.
120. Hirohata S, Arinuma Y, Yanagida T, Yoshio T. Blood-brain barrier damages and intrathecal synthesis of anti- N-methyl-D-aspartate receptor NR2 antibodies in diffuse psychiatric/neuropsychological syndromes in systemic lupus erythematosus. Arthritis Res Ther. 2014;16(2):R77.
121. Arinuma Y, Yanagida T, Hirohata S. Association of cerebrospinal fluid anti-NR2 glutamate receptor antibodies with diffuse neuropsychiatric systemic lupus erythematosus. Arthritis Rheum. 2008;58(4):1130-5.
122. 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.
123. Omdal R, Brokstad K, Waterloo K, Koldingsnes W, Jonsson R, Mellgren SI. Neuropsychiatric disturbances in SLE are associated with antibodies against NMDA receptors. Eur J Neurol. 2005;12(5):392-8.
124. Kozora E, West SG, Maier SF, Filley CM, Arciniegas DB, Brown M, et al. Antibodies against N-methyl-D-aspartate receptors in patients with systemic lupus erythematosus without major neuropsychiatric syndromes. J Neurol Sci. 2010;295(1-2):87-91.
125. Petri M, Naqibuddin M, Carson KA, Wallace DJ, Weisman MH, Holliday SL, et al. Depression and cognitive impairment in newly diagnosed systemic lupus erythematosus. J Rheumatol. 2010;37(10):2032-8.
126. Freeman JM. Rasmussen's syndrome: progressive autoimmune multi-focal encephalopathy. Pediatric neurology. 2005;32(5):295-9.
127. Takahashi Y, Mori H, Mishina M, Watanabe M, Kondo N, Shimomura J, et al. Autoantibodies and cell-mediated autoimmunity to NMDA-type GluRepsilon2 in patients with Rasmussen's encephalitis and chronic progressive epilepsia partialis continua. Epilepsia. 2005;46 Suppl 5:152-8.
128. Steiner J, Walter M, Glanz W, Sarnyai Z, Bernstein HG, Vielhaber S, et al. Increased prevalence of diverse N-methyl-D-aspartate glutamate receptor antibodies in

patients with an initial diagnosis of schizophrenia: specific relevance of IgG NR1a antibodies for distinction from N-methyl-D-aspartate glutamate receptor encephalitis. JAMA psychiatry. 2013;70(3):271-8.
129. Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. Lancet neurology. 2011;10(1):63-74.
130. Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. J Neurosci. 2010;30(17):5866-75.
131. Appenzeller S, Pike GB, Clarke AE. Magnetic resonance imaging in the evaluation of central nervous system manifestations in systemic lupus erythematosus. Clin Rev Allergy Immunol. 2008;34(3):361-6.
132. Sibbitt WL, Jr., Brooks WM, Kornfeld M, Hart BL, Bankhurst AD, Roldan CA. Magnetic resonance imaging and brain histopathology in neuropsychiatric systemic lupus erythematosus. Semin Arthritis Rheum. 2010;40(1):32-52.
133. Fazekas F. Magnetic resonance signal abnormalities in asymptomatic individuals: their incidence and functional correlates. Eur Neurol. 1989;29(3):164-8.
134. Harboe E, Beyer MK, Greve OJ, Goransson LG, Tjensvoll AB, Kvaloy JT, et al. Cerebral white matter hyperintensities are not increased in patients with primary Sjogren's syndrome. Eur J Neurol. 2009;16(5):576-81.
135. Sibbitt WL, Jr., Sibbitt RR, Brooks WM. Neuroimaging in neuropsychiatric systemic lupus erythematosus. Arthritis Rheum. 1999;42(10):2026-38.
136. Muscal E, Traipe E, de Guzman MM, Myones BL, Brey RL, Hunter JV. Cerebral and cerebellar volume loss in children and adolescents with systemic lupus erythematosus: a review of clinically acquired brain magnetic resonance imaging. J Rheumatol. 2010;37(8):1768-75.
137. Steens SC, Bosma GP, ten Cate R, Doornbos J, Kros JM, Laan LA, et al. A neuroimaging follow up study of a patient with juvenile central nervous system systemic lupus erythematosus. Ann Rheum Dis. 2003;62(6):583-6.
138. Kozora E, West SG, Kotzin BL, Julian L, Porter S, Bigler E. Magnetic resonance imaging abnormalities and cognitive deficits in systemic lupus erythematosus patients without overt central nervous system disease. Arthritis Rheum. 1998;41(1):41-7.
139. Ainiala H, Dastidar P, Loukkola J, Lehtimaki T, Korpela M, Peltola J, et al. Cerebral MRI abnormalities and their association with neuropsychiatric manifestations in SLE: a population-based study. Scand J Rheumatol. 2005;34(5):376-82.
140. Appenzeller S, Vasconcelos Faria A, Li LM, Costallat LT, Cendes F. Quantitative magnetic resonance imaging analyses and clinical significance of hyperintense white matter lesions in systemic lupus erythematosus patients. Ann Neurol. 2008;64(6):635-43.
141. Coates T, Slavotinek JP, Rischmueller M, Schultz D, Anderson C, Dellamelva M, et al. Cerebral white matter lesions in primary Sjogren's syndrome: a controlled study. J Rheumatol. 1999;26(6):1301-5.
142. Petri M, Naqibuddin M, Carson KA, Wallace DJ, Weisman MH, Holliday SL, et al. Brain magnetic resonance imaging in newly diagnosed systemic lupus erythematosus. J Rheumatol. 2008;35(12):2348-54.

143. Appenzeller S, Bonilha L, Rio PA, Min Li L, Costallat LT, Cendes F. Longitudinal analysis of gray and white matter loss in patients with systemic lupus erythematosus. Neuroimage. 2007;34(2):694-701.
144. 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.
145. Gitelman DR, Klein-Gitelman MS, Ying J, Sagcal-Gironella AC, Zelko F, Beebe DW, et al. Brain morphometric changes associated with childhood-onset systemic lupus erythematosus and neurocognitive deficit. Arthritis Rheum. 2013;65(8):2190-200.
146. Alexander EL, Beall SS, Gordon B, Selnes OA, Yannakakis GD, Patronas N, et al. Magnetic resonance imaging of cerebral lesions in patients with the Sjogren syndrome. Ann Intern Med. 1988;108(6):815-23.
147. Pierot L, Sauve C, Leger JM, Martin N, Koeger AC, Wechsler B, et al. Asymptomatic cerebral involvement in Sjogren's syndrome: MRI findings of 15 cases. Neuroradiology. 1993;35(5):378-80.
148. Alhomoud IA, Bohlega SA, Alkawi MZ, Alsemari AM, Omer SM, Alsenani FM. Primary Sjogren's syndrome with central nervous system involvement. Saudi Med J. 2009;30(8):1067-72.
149. Tzarouchi LC, Tsifetaki N, Konitsiotis S, Zikou A, Astrakas L, Drosos A, et al. CNS involvement in primary Sjogren Syndrome: assessment of gray and white matter changes with MRI and voxel-based morphometry. AJR Am J Roentgenol. 2011;197(5):1207-12.
150. Appenzeller S, Carnevalle AD, Li LM, Costallat LT, Cendes F. Hippocampal atrophy in systemic lupus erythematosus. Ann Rheum Dis. 2006;65(12):1585-9.
151. Appenzeller S, Rondina JM, Li LM, Costallat LT, Cendes F. Cerebral and corpus callosum atrophy in systemic lupus erythematosus. Arthritis Rheum. 2005;52(9):2783-9.
152. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage. 2000;11(6 Pt 1):805-21.
153. Whitwell JL. Voxel-based morphometry: an automated technique for assessing structural changes in the brain. J Neurosci. 2009;29(31):9661-4.
154. 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 Rheum. 1982;25(11):1271-7.
155. Daniels TE, Silverman S, Jr., Michalski JP, Greenspan JS, Sylvester RA, Talal N. The oral component of Sjogren's syndrome. Oral surgery, oral medicine, and oral pathology. 1975;39(6):875-85.
156. Wechsler D. WMS-R: Wechsler Memory Scale-Revised : Manual: Harcourt Brace Jovanovich; 1987.
157. Halstead WC. Brain and intelligence. Chicago: University of Chicago Press; 1947.
158. Lezak MD. Memory I: Tests. In: Lezak MD, editor. Neuropsychological assessment. Fourth ed. Oxford: Oxford University Press; 2004. p. 414-79.
159. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4:561-71.

160. Beck AT, Beamesderfer A. Assessment of depression: the depression inventory. Mod Probl Pharmacopsychiatry. 1974;7(0):151-69.
161. Whitwell JL, Crum WR, Watt HC, Fox NC. Normalization of cerebral volumes by use of intracranial volume: implications for longitudinal quantitative MR imaging. AJNR American journal of neuroradiology. 2001;22(8):1483-9.
162. Ashburner J, Friston KJ. Unified segmentation. Neuroimage. 2005;26(3):839-51.
163. Scheltens P, Barkhof F, Leys D, Pruvo JP, Nauta JJ, Vermersch P, et al. A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. J Neurol Sci. 1993;114(1):7-12.
164. Steup-Beekman G, Steens S, van Buchem M, Huizinga T. Anti-NMDA receptor autoantibodies in patients with systemic lupus erythematosus and their first-degree relatives. Lupus. 2007;16(5):329-34.
165. Grieve SM, Korgaonkar MS, Koslow SH, Gordon E, Williams LM. Widespread reductions in gray matter volume in depression. NeuroImage Clinical. 2013;3:332-9.
166. Chumbley JR, Friston KJ. False discovery rate revisited: FDR and topological inference using Gaussian random fields. Neuroimage. 2009;44(1):62-70.
167. Sowell ER, Peterson BS, Thompson PM, Welcome SE, Henkenius AL, Toga AW. Mapping cortical change across the human life span. Nat Neurosci. 2003;6(3):309-15.
168. Faust TW, Chang EH, Kowal C, Berlin R, Gazaryan IG, Bertini E, et al. Neurotoxic lupus autoantibodies alter brain function through two distinct mechanisms. Proc Natl Acad Sci U S A. 2010;107(43):18569-74.
169. Nopoulos P, Flaum M, O'Leary D, Andreasen NC. Sexual dimorphism in the human brain: evaluation of tissue volume, tissue composition and surface anatomy using magnetic resonance imaging. Psychiatry Res. 2000;98(1):1-13.
170. Lemaitre H, Crivello F, Grassiot B, Alperovitch A, Tzourio C, Mazoyer B. Age- and sex-related effects on the neuroanatomy of healthy elderly. Neuroimage. 2005;26(3):900-11.

10. Errata

11. Supplementary

11.1

1997 Update of the 1982 American College of Rheumatology Revised Criteria for Classification of Systemic Lupus Erythematosus (24)

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 physician
5. Arthritis	Nonerosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	1. Pleuritis – convincing history of pleuritic pain or rubbing heard by a physician or evidence of pleural effusion or 2. Pericarditis – documented by electrocardigram or rub or evidence of pericardial effusion
7. Renal disorder	1. Persistent proteinuria greater than 0.5 grams per day or greater than 3+ if quantitation not performed or 2. Cellular casts – may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic disorder	1. Seizures – in the absence of offending drugs or known metabolic derangements; e.g. uremia, ketoacidosis, or electrolyte imbalance or 2. Psychosis – in the absence of offending drugs or known metabolic derangements, e.g. uremia, ketoacidosis, or electrolyte imbalance
9. Hematologic disorder	1. Hemolytic anemia – with reticulocytosis or 2. Leukopenia – less than 4,000/mm3 on ≥ 2 occasions or 3. Lymphopenia – less than1,500/ mm3 on ≥ 2 occasions or 4. Thrombocytopenia – less than100,000/ mm^3 in the absence of offending drugs
10. Immunologic disorder	1. Anti-DNA: antibody to native DNA in abnormal titer or 2. Anti-Sm: presence of antibody to Sm nuclear antigen or 3. Positive finding of antiphospholipid antibodies on: 1. an abnormal serum level of IgG or IgM anticardiolipin antibodies, 2. a positive test result for lupus anticoagulant using a standard method, or 3. a false-positive test result for at least 6 months confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
11. Antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs

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 or observation

11.2

American-European Consensus Group Classification Criteria for Sjögren's Syndrome (34)

I. Ocular symptoms: A positive response to at least one of the following questions:

1. Have you had daily, persistent, and troublesome dry eyes for more than 3 months?
2. Do you have a recurrent sensation of sand or gravel in the eyes?
3. Do you use tear substitutes more than 3 times a day?

II. Oral symptoms: A positive response to at least one of the following questions:

1. Have you had a daily feeling of dry mouth for more than 3 months?
2. Have you had recurrent or persistently swollen salivary glands as an adult?
3. Do you frequently drink liquids to aid in swallowing dry food?

III. Ocular signs, that is, objective evidence of ocular involvement, defined as a positive result for at least one of the following two tests:

1. Schirmer's I test, performed without anesthesia (<5 mm in 5 minutes)
2. Rose Bengal score or other ocular dye score (>4 according to van Bijsterveld's scoring system)

IV. Histopathology: Focal lymphocytic sialoadenitis in minor salivary glands (obtained through normal-appearing mucosa) evaluated by an expert histopathologist, with a focus score >1, defined as a number of lymphocytic foci (adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes) per 4 mm^2 of glandular tissue.

V. Salivary gland involvement: Objective evidence of salivary gland involvement defined by a positive result for at least one of the following diagnostic tests:

1. Unstimulated whole salivary flow (<1.5 ml in 15 minutes)
2. Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitary, or destructive pattern), without evidence of obstruction in the major ducts
3. Salivary scintigraphy showing delayed uptake, reduced concentration, and/or

a. delayed excretion of tracer

VI. Autoantibodies: Presence in the serum of the following autoantibodies:

1. Antibodies to Ro (SSA) or La (SSB) antigens, or both

For primary SS:

In patients without any potentially associated disease, primary SS may be defined as follows:

a. The presence of any 4 of the 6 items is indicative of primary SS, as long as either item IV (Histopathology) or VI (Serology) is positive
b. The presence of any 3 of the 4 objective criteria items (that is, items III, IV, V, or VI)
c. The classification tree procedure represents a valid alternative method for classification, although it should be more properly used in a clinical- epidemiological survey

Exclusion criteria:

1. Past head and neck radiation treatment
2. Hepatitis C infection
3. Acquired immunodeficiency syndrome (AIDS)
4. Pre-existing lymphoma
5. Sarcoidosis
6. Graft versus host disease
7. Use of anticholinergic drugs (since a time shorter than 4-fold the half life of the drug)

11.3

Neuropsychiatric syndromes in systemic lupus erythematosus (31)

Central nervous system

Aseptic meningitis
Cerebrovascular disease
Demyelinating syndrome
Headache (including migraine and benign intracranial hypertension)
Movement disorder (chorea)
Myelopathy
Seizure disorders
Acute confusional state
Anxiety disorder
Cognitive dysfunction
Mood disorder
Psychosis

Peripheral nervous system

Acute inflammatory demyelinating polyradiculoneuropathy (Guillain-Barré syndrome)
Autonomic disorder
Mononeuropathy, single/multiplex
Myasthenia gravis
Neuropathy, cranial
Plexopathy
Polyneuropathy