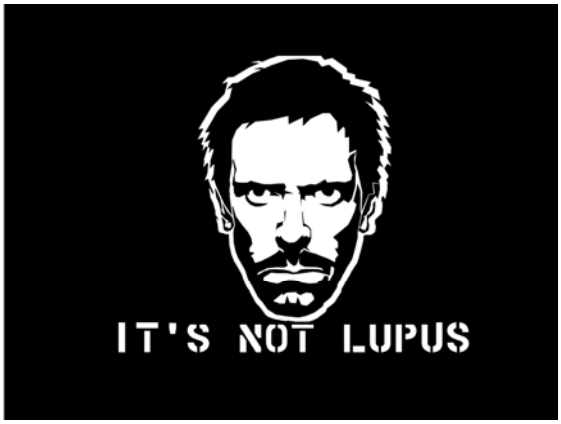
Phospholipid antibodies in the cerebrospinal fluid and serum, in SLE patients with suspected CNS involvement and associations with depression and cognitive impairment.



Phospholipid antibodies in the cerebrospinal fluid and serum, in SLE patients with suspected CNS involvement and associations with depression and cognitive impairment.

Master thesis in Medicine

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Abstract

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Introduction

A broad spectrum of neuropsychiatric symptoms is common among SLE patients (NPSLE) as well as patients affected by Anti-phospholipid-syndrome (APS). Indicating a connection between neuropsychiatric illness and presence of anti phospholipid antibodies (aPLs) in patient's sera.

Aims

Examine if it’s possible to improve diagnosis of NPSLE patients by testing cerbrospinalfluid (CSF) for cardiolipin and β2-glycoprotein1 IgG antibodies. Investigate if aPLs presence in CSF and/or serum associates with cognitive impairment and depression.

Methods

CSF was collected from 51 SLE patients with neuropsychiatric involvement. Patient's serological values of phospholipid antibodies (LA, aCL, aβ2GP1) were obtained by retrospective inspection of medical records. Comprehensive screening of depression and cognitive impairment was performed. Chi square test and Fischer's exact test was used to calculate association between aPLs positivity, depression and cognitive impairment.

Results

None of the patients were CSF positive for aCL or aβ2GP1. 27% were positive in serum for at least one aPLs. 59% suffered from depression. 45% suffered from cognitive impairment. Most common cognitive impairment was linguistic designation difficulties. Association between aPLs positivity and impaired linguistic designation was significant when including limit values for β2GP1 ($p= 0.032$). There was no association between aPLs positivity and depression or global cognitive impairment ($p >0.05$)

Discussion and conclusions

Diagnosis was not improved by testing CSF for aCL and aβ2GP1 IgG antibodies. Depression and cognitive impairment is common under diagnosed in NPSLE patients. It's therefore important to continuously screen SLE patients for these conditions, regardless of aPLs positivity, although an association between serological aPLs presence and impaired linguistic designation is possible.

Introduction

SLE

Systemic Lupus Erythematosus SLE, often referred to as lupus, Latin for wolf, is a chronic systemic inflammatory autoimmune disease that can affect virtually all body organs. Disease activity and severity varies significant between affected individuals. Most frequently affected organs are joints, skin, kidneys, serous membrane (pleura, pericardium), blood cells (hemolytic anemia, leukopenia, lymphopenia, thrombocytopenia), blood vessels and the central and/or peripheral nervous system. SLE is characterized by alternating periods of active and latent disease. Associated symptoms such as fatigue, weight loss, fever, alopecia and sensitivity to sunlight are common. Diagnosis is based on clinical symptoms, -involving at least two different organ-systems, with the support of associated laboratory findings. Important laboratory findings is the presence of autoantibodies against DNA, nuclear material and other proteins, including anti-phospholipid antibodies (1). Lupus systemic nature and its wide spectrum of clinical symptoms, which often mimic those of other diseases, make Lupus hard to define and diagnose. A diagnostic aid is the criteria of the American College of Rheumatology updated 1982 and 1997. The ACR criteria are standard for the definition of SLE in clinical studies (2, 3) (Table 1).

Table 1. *1997 Update on revised SLE classification criteria defined by American collage of rheumatology***.** *Four of eleven criteria are mandatory for SLE classification in clinical studies. The criteria have 96% clinical sensitivity and specificity.* (2, 3)

Criterion:	Definition:
1. Malar rash	Fixed malar erythema over the malar eminence, flat or raised, tending to spare the nasolabial folds.
2. Discoid rash	Raised erythematosus patches with adherent keratotic scaling and follicular plugging's, atrophic scaring may occur in older lesions.
3. Photosensitivity	Skin rash as un unusual reaction after exposure to sunlight.
4. Oral ulcers	Oral or nasopharyngeal ulcerations
5. Non-erosive arthritis	Involving two or more peripheral joints, characterized by tenderness, swelling, or effusion.
6. Serositis	Pleuritis or pericarditis
7. Renal disorder	Persistent proteinuria > 0.5 grams per day or > than 3+ if quantitation not performed *OR* Cellular casts--may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurological disorder	Seizures--in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance *OR* Psychosis--in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance

9. Hematologic disorder	Hemolytic anemia--with reticulocytosis *OR* Leukopenia--< 4,000/mm^3 on ≥ 2 occasions *OR* Lyphopenia--< 1,500/ mm^3 on ≥ 2 occasions *OR* Thrombocytopenia--<100,000/ mm^3 in the absence of offending drugs
10. Immunologic disorder	Anti-DNA: antibody to native DNA in abnormal titer *OR* Anti-Sm: presence of antibody to Sm nuclear antigen *OR* Positive finding of antiphospholipid antibodies on: An abnormal serum level of IgG or IgM anticardiolipin antibodies, A positive test result for lupus anticoagulant using a standard method, or A false-positive test result for at least 6 months confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
11. Positive 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

Epidemiology

SLE has a strong female dominance and about 90 % of the affected is women in reproductive age of 15-40 years. However, the disease can affect both sexes and all ages. The average age of diagnosis is between 35-45 years. Worldwide disease incidence varies approximately between 1-11/100 000 of the population a year. The variation is depending on the study design, the method used for diagnostic, time span and the country where the study was preformed (1). Earlier Swedish studies performed between 1981-1991 shows an overall incidence of approximately 4.8/100 000 and a prevalence about 36-68/100 000 (4-6).

Pathogenicity and treatment

Disease pathology is highly complex, multifactorial and not yet fully understood. Dysfunction in the cell mediated and the humoral mediated immune system are believed to contribute to disease development. Errors in apoptotic pathways and altered antigen presentation as well as abnormalities in B and T cells development and function, leads to production of inflammatory cytokines and autoantibodies against nuclear acid and its binding proteins. Immune complex is formed when autoantibodies binds to self-tissue antigens. Accumulation of these immune complexes wrongly activates the complement system and causes multi organ inflammation and damage (7, 8). Lupus can most likely be triggered by both environmental and genetic factors. A first-degree relative with one or more autoimmune diseases increase the risk of disease development (9). SLE disease development concordance is 25% in monozygotic twins compared to 2 % in dizygotic twins (10). Established environmental risk factors are endogen and exogenous exposure to estrogens, especially 16alfahydroxyesteron, exposure to UV light, current smoking and medications e.g. procainamide, hydralazine. Lupus is treated with immunosuppressive therapy, anti-inflammatory drugs, anti-malarias, and steroids (1).

Late complications

Effective treatment and better diagnostic methods have reduced mortality associated with acute disease activity. However, the increased mortality rate among SLE patients is today mainly caused by late complications in form of cardiovascular disease (CVD). SLE patients has an increased susceptibility to develop atherosclerosis, often affecting the coronary arteries and in addition of traditional risk factors (11-13). A Swedish cohort from Gustafson et al (13) found aPLs presence a predictive risk factor for cardio vascular mortality (CVM) in SLE patients. A bright spot is that treatment with anti malaria agent. Besides the drugs direct effect on SLE activity, it also have vascular protective features, as they are believed to normalize lipid and glucose level and decreases the risk of thrombosis (1).

NPSLE

Neuropsychiatric Systemic Lupus Erythematosus, NPSLE, is when the systemic disease, focal and/or diffuse affects the central and/or the peripheral nervous system. Neuropsychiatric manifestations are common in SLE patients and its frequency varies between 12-95%. The broad variation in frequency is mainly due to differences in study design and a diagnostic inclusion criterion's used to define NPSLE (1). The Neuropsychiatric symptoms vary from mild cognitive dysfunction, anxiety and depression, to manifestations such as stroke, psychosis and seizures (14). In 1999 the American College of Rheumatology (ACR) ad Hoc committee developed an extensive standardize nomenclature system that define 19 NPSLE syndromes (15) (Table 2). The ACR criteria are today standard for describing NP events in clinical studies (1). Cognitive dysfunction and mood disorders are two of the most frequent NP-syndromes affecting approximately 20% of NPSLE patients (1, 14, 16, 17).

Table 2. *Neuropsychiatric syndromes in SLE defined by the American collage of rheumatology* (18).

1. Acute Confusional State	11. Mood disorders
2. Acute inflammatory demyelinating Polyradiculoneuropathy (Gillian Barre syndrome)	12. Movement disorders (Chorea)
3. Anxiety Disorder	13. Myasthenia Gravis
4. Aseptic Meningitis	14. Myelopathy
5. Autonomic disorder	15. Neuropathy, Cranial
6. Cerebrovascular disease	16. Plexopathy
7. Cognitive dysfunction	17. Polyneuropathy
8. Demyelinating syndromes	18. Psychosis
9. Headache	19 Seizures and Seizure Disorders
10. Mononeuropathy single/multiplex	

Pathogenesis

NPSLE pathogenicity remains unclear and not yet fully understood. Microvasculopathy, thrombosis, autoantibodies, inflammatory mediators and blood brain barrier dysfunction are believed to contribute to disease development (19). Growing evidence supports that several different autoantibodies, especially when they gain access to CSF and brain tissue play an important role in disease development. A frequently cited study performed by Giorgio et al, (20) showed that Lupus antigens (anti-DNA-antibodies) cross react with DNA receptors as well as neural glutamate binding- N-methyl-D-aspartate (NMDA) receptor subunits, NR2a and NR2b. Auto binding to NMDA induces neuronal apoptosis, provides access to CSF and mediates non-thrombotic and non- vascular abnormalities in the central nervous system. NMDA subunit antibodies and its neurotoxicity were later observed in CSF from a SLE patient with cognitive impairment. Kowal et al. (21, 22) proved that an intracranial injection of anti NMDA receptor antibodies in mice caused apoptosis of hippocampus and causes cognitive impairment. Results from Yoshio et al. (23) indicate that glutamate NR2 rec antibodies in CSF may be more accurate then serum samples when diagnosing NPSLE. Several other studies show a strong association between NPSLE and persistent presence of phospholipid antibodies (aPLs) in serum. Elevated serum titers of aPLs are believed to be a risk factor for NPSLE development (14).

Diagnostic methods and treatment

It`s often a long process before a patient gets diagnosed with NPSLE. First the physician needs to exclude and treat other non-SLE related causes. Then clarify if the neuropsychiatric symptoms are due to SLE-mediated organ dysfunction or infection, medication side effects or metabolic abnormalities. Clinical examination, laboratory analyzes of serum and CSF samples as well as neuroimaging, preferably MRI are helpful tools (14). CSF abnormalities in NPSLE patients include; pleocytos and/or mild elevated protein levels, increased intrathecal synthesis of immunoglobulin's and/or oligoclonal bands, elevated levels of pro-inflammatory cytokines iL6, IL8 as well as elevated levels of astroglial degradation products (24-27). Although there is no single specific test method to diagnose NPSLE, there is a huge need for such a method. NPSLE is considered one of the most serious manifestations of SLE, leading to both increased morbidity and mortality in SLE patients (28-31). Treatment depends on pathogenicity. Symptomatic treatment is used in addition when necessary. Primary NPSLE without presence of aPLs is treated with immunosuppressive therapy, manly cyclophosphamide (14, 19).

Anti phospholipid antibodies and clinical features

Phospholipids function as building blocks in various organelles and cellular membranes. Phospholipid antibodies (aPLs) can react with complexes of phospholipids and plasma proteins such as Beta2 Glycoprotein1 (aβ2GP1), prothrombin, annexin V, protein C and protein S. aPLs can mediate thrombosis development by inhibit natural anti coagulant system (protein C and S), impair fibrinolysis or trough direct impact on cellular functions. In addition to thrombosis development aPLs may also bind directly to brain tissue cells and endothelial cells. The reactions trigger a cascade of neuroinflammatory responses that undermine the protective function of the blood brain barrier. (1). A different isotype of aPLs was found in CSF, compared to serum in a study involving patients suffering from psychosis. This finding might indicate a possible intrathekal production of phospholipid antibodies during neuropsychiatric disease (32). Stojanovich L et al. (33) showed an association between the positivity of aPLs and several non-thrombotic neurological manifestations.

aPLs are present in approximately 1-5% of the general asymptomatic healthy population, with a higher frequency among the elderly population (34, 35). Infections, neoplasm, active vaccination and drug therapies are known to cause elevation of these autoantibodies (34, 36-39). Anti phospholipid syndrome (APS) is when persistent presence of aPLs causes clinical symptoms, defined as one or more event of vascular thrombosis and/or pregnant morbidity. aPLs used to diagnose APS are Lupus anticoagulant (LA), anti-cardiolipin (aCL) and anti- Beta2Glycoprotein1 (aB2GP1) (40).

Anti Phospholipid Syndrome (APS)

APS is when persistent presence of aPLs causes clinical symptoms. It is an autoimmune disease that can cause thrombosis in both arteries and veins and in all vessel sizes and organs. It can also cause thrombocytopenia, severe pregnancy morbidity and neurological disease. Primary APS, independent of other diseases occur, but APS is often linked to other autoimmune diseases particularly SLE (1). Approximately 36% of SLE patients suffer from secondary APS (41). Diagnosis is based on clinical symptoms as vascular thrombosis and/or pregnancy morbidity and positive serological test for at least one phospholipid antibody: Positive test for lupus anticoagulants (LA), medium or high titers for Beta2 glycoprotein 1 (aβ2GP1) IgG or IgM antibodies, or medium or high titers of Cardiolipin (aCL) IgG or IgM antibodies, with a positive persistence over 12 weeks (40, 42, 43). Estimated incidence of APS is about 5/100.000 persons a year and the prevalence varieties between 40-50/100.000 (40). Neuropsychiatric illness is common in APS patients (44, 45). APS related neuropsychiatric symptoms vary from transient ischemic attack (TIA) and stroke to epileptic seizures, depression, impaired cognition, headaches and psychosis (41, 46). Anti phospholipid syndrome with clinical manifestations is treated with life long anti coagulant therapy, mainly Warfarin (47-49).

Scientific issue

A variety of neurological and psychiatric manifestations are linked to the presence of aPLs in the patient's sera. There is a strong comorbidity between SLE and APS and both diseases can cause neuropsychiatric symptoms (50-52). With current available diagnostic methods it’s impossible to determinate the true pathogenesis of neuropsychiatric symptoms in a SLE patient especially when suffering from secondary APS. Treatment is therefore often directed against both diseases, with anticoagulation- and immunosuppressive therapy. Another major difficulty is to discern whether the cognitive impairment and/or depression are due to immunological reactions in the brain caused by antibodies, or whether it is a result of living with a difficult chronic disease.

Aims

1. Examine if it's possible to improve diagnosis of SLE patients with suspected CNS involvement and possible serological aPLs positivity (LA, aCL, β2GP1) by testing CSF for Cardiolipin and β2-glycoprotein1 IgG antibodies.

2. Investigate if the CSF-titers and/or serum titers of anti-phospholipid antibodies in NPSLE patients associates with cognitive impairment and/or depression.

Materials and Methods

Study population

Cerebrospinal fluid (CSF) samples were collected from 51 consent SLE patients at Sahlgrenska University hospital, rheumatology clinic in Gothenburg, Sweden between 2011-2013. All patients included in the study were previously diagnosed with SLE according to at least 4 ACR/SLE criteria and suffered from CNS involvement according to at least one ACR/NPSLE criteria. Of these, 16 patients suffered from depression and 10 suffered from cognitive impairment, according to ACR at the start of the study. The participants, 6 men and 44 women were aged 20-71 years old, the average age was 40 years old and had an average disease duration of 8,5 years. Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and Systemic Lupus International Collaborating Clinics (SLICC) were used to estimate the SLE disease activity and chronic damage.

Serum samples

Serum samples of phospholipid antibodies (LA, aCL, aβ2GP1) were collected and analyzed earlier for diagnostic purpose during the 51 patient's hospitalization. Serum test result and titers were obtained by retrospective inspection of medical records.

CSF laboratory analyses

The collected CSF samples were kept frozen at -70 C until assayed, according to customary routines used at SU clinical immunology and transfusion medicine. CSF analyses of aCL and aβ2GP1 were performed with an automatic enzyme-linked immunosorbent assay (ELISA) based method (Alegria from Orgentec Diagnostica GmbH). All analyses were performed in accordance with the manufacturer's instructions.

The Sensotronic Memorized Calibration (SMC) technology is a method based on an indirect enzyme immunoassay reaction. The test consists of 8 barcode labeled strips. Each strip is designed for one antigen and consists of enzyme conjugate, enzyme substrate, sample dilution buffer and a quality check. 10µl CSF sample was added to each strip. The samples were incubated, washed with conjugate and washed again. Substrate was added and triggered a hydrolyzing process. The test result was measured spectrophotometrically, as a blue color shift according to the amount of antibodies in the sample occurred.

Positive controls for aCL and aβ2GPI sera were run in the same assay as CSF samples to confirm specificity of the results. The manufactures reference range and limit values based on serum samples was used to determinate aCL and aβ2GP1 positivity.

Reference range

aCL IgG units/ml: Values below 10 units /ml was considered negative and values at 10 units/ml and over, were considered positive (53).

aβ2GP1 IgG units/ml: Values below 5 units/ml were considered negative, values between 5-9 units/ml were considered limit values and values at 10 units/ml and over were considered positive (53).

Depression and cognitive impairment

Screening of depression and cognitive impairment was performed by experienced nursing staff at Sahlgrenska University hospital. To detect possible depression, study protocol included two depression surveys; the Montgomery–Åsberg Depression Rating Scale (MADRS) and Patient Health Questionnaire-9 (PHQ-9) and one general screening for neuropsychiatric disease, The Mini International Neuropsychiatric Interview (MINI). The patients were classified as depressed if they were rated depressed (mild-sever) at one of these three screening tests. To detect possible cognitive impairment, we used the cognitive screening battery (KSB in Swedish), correspondent to the cognitive assessment battery (CAB in English). The test is designed in accordance with the recommendations of the American Academy of Neurology (AAN). The cognitive assessment battery is a sensitive and specific test that measures performances in several cognitive functions as: Speed and attention, episodic memory and learning, visuospatial function, language and executive function (54). Test results were assessed on the basis of age-averages normal value for healthy individuals. Performances of 1.5 standard deviations (SD) below average age were classed as pathological, in accordance with test protocol (55). Patients who scored 1.5 SD below average age in at least one of the 6 cognitive tests were considerate cognitive impaired and referred to as global cognitive impaired. The subject's cognitive impairment was classified as mild, moderate or severe, based on the number of impaired domains (1-.2, mild; 3-4, moderated; severe >5).

Statistical methods

Statistical Package for Social Sciences, SPSS (version 22) was used to calculate the statistic results.

The participants were divided in to two subgroups according to if they were serum positive for at least one of the tree aPLs, or aPLs serum negative. Calculations including limit values for aβ2GP1 in the positive group respectively in the negative group were performed.

Chi square test and Fischer's exact test was used to calculate possible association between aPLs serum positivity, depression and cognitive impairment. Two sided P values <0.05 was considered significant.

Ethics

Our study is a part of a larger study that examines neuropsychiatric symptoms in SLE patients. Ethical license number Dnr 433-11, approved 2011-07-05. Study participation is completely anonymous and voluntary. All participants are given both verbal and written information about study aims and design prior consent. Participants may at any time, without explanation, terminate their participation in the study, and/or request that their samples be destroyed.

Results

Our aim was to examine if CSF phospholipid antibody testing could improve the diagnosis of NPSLE patients. We also wanted to see if phospholipid antibodies in NPSLE patients were associated with cognitive impairment and/or depression.

CSF

None of the patients were CSF positive for phospholipid antibodies (aCL or aβ2GP1). All CSF samples analyzed in the study were well below the serum threshold limit values.

Serum

Retrospective review of medical records showed that; 14 (27%) of the 51 NPSLE patients were aPLs positive in serum. 11 (22%) were positive for Cardiolipin antibodies (aCL). 7 (14%) were positive for- and 3 (6%) hade limit values of Beta2-glycoprotein antibodies (aβ2GP1). 9 (18%) were positive for Lupus anticoagulation (LA). 5 (10%) patients were positive for 2 antibodies. 3 (6%) of the patients were positive for all three antibodies, so-called triple positive (Table 3).

Table 3 *Frequency of serological aPLs positivity and limit values in study population.*

	aPLs	aCL	aβ2GP1	LA	Double	Triple
Positive	14 (27%)	11 (22%)	7 (14%)	9 (18%)	5 (10%)	3 (6%)
Limit			3 (6%)			

Cognitive impairment

23 patients (45%) suffered from some sort of cognitive impairment, compared to 10 (20%) cognitive dysfunctional patients diagnosed according to ACR criteria at study entry (table 6). 12 patient (23%) suffered from mild cognitive impairment and 11 patients (22%) from moderate cognitive impairment, none of the patients suffered from sever cognitive impairment. Most common cognitive impairments were; linguistic designation- and speed and attention difficulties (table 4). A higher percentage of the aPLs positive patients suffered from some type of cognitive impairment compared to aPLs negative patients, but the difference was not significant (Table 5 and 6). The association between aPLs positivity and impaired linguistic designation was significant when including limit values for B2GP1 (p=0.032) (Table 7).

Table 4 *Screened frequency of Cognitive impairment in study population according to the Cognitive assessment battery (CAB).*

	Total n=51	aPLs positive n=16	aPLs negative n=35
Learning and episodic memory	4 (8%)	1 (6%)	3 (9%)
Speed attention	12 (23%)	5 (31%)	7 (20%)
Linguistic designation	16 (31%)	8 (50%)	8 (23%)
Spatial functions	5 (10%)	1 (6%)	4 (8%)
Linguistic functions (token):	6 (12%)	3 (19%)	3 (6%)
Executive function	10 (20%)	4 (25%)	6 (12%)

Depression

30 patients 59% suffered from depression (mild-sever) according to at least one screening survey, compared to 16 (31%) patients diagnosed with mood disorder according to ACR criteria at study entry (Table 6). There was no significant association between depression and aPLs positivity. A higher percentage of aPLs negative patients suffered from depression compared to aPLs positive patients, but the difference was not significant (table 5 and 6).

Table 5. *Clinical characteristic of study NPSLE patients with and without serological aPLs positivity, including limit values for aβ2GP1in the positive group.*

	aPLs positive n=16	aPLs negative n=35
Average age	37,8	41,8
Gender	16 females 0 males	29 females 6 males
Average years with SLE	11,6	9,9
Most frequent ACR/NPSLE syndrome at study entry	Mood disorders: 5 (31%) Headache: 3 (19%) CD*: 3 (19%) CVD*: 3 (19%)	Mood disorders: 11 (31%) Headache: 8 (22%) CD*: 7 (20%) Cranial Neuropathy: 5 (14%)
Average SLE damage index (SLICC)	1,3	1,4
Average Disease activity (SLEDAI)	8,6	8,6
Number of patients with cognitive impairment (CAB)	9 (56%)	14 (40%)
Number of patients with depression (MADRAS, PHQ9, MINI)	8 (50%)	22 (63%)

**Cognitive dysfunction (CD) *Cerebrovascular disease (CVD)*

Table 6. *Shows the number of patients with Mood disorder or cognitive dysfunction according to ACR criteria's compared to the number of patients with depression or cognitive impairment found in our screening.*

	Total n= 51	aPLs positive n=16	aPLs negative n=35
ACR Mood disorder *	16 (31%)	5 (31%)	12 (34%)
Screened number of patients with depression	30 (59%)	8 (50%)	22 (63%)
ACR Cognitive dysfunction *	10 (20%)	3 (19%)	7 (20%)
Screened number of patients with cognitive impairment	23 (45%)	9 (56%)	14 (40%)

* Number of patients diagnosed according to ACR at study entry.

Our screening showed a higher prevalence of cognitive impairment and depression in the study population than expected, when compared to patients diagnosed with cognitive dysfunction and mood disorder according to ACR/NPSLE criteria at study entry. Indicating that depression and cognitive impairment is under diagnosed among SLE patients with CNS involvement.

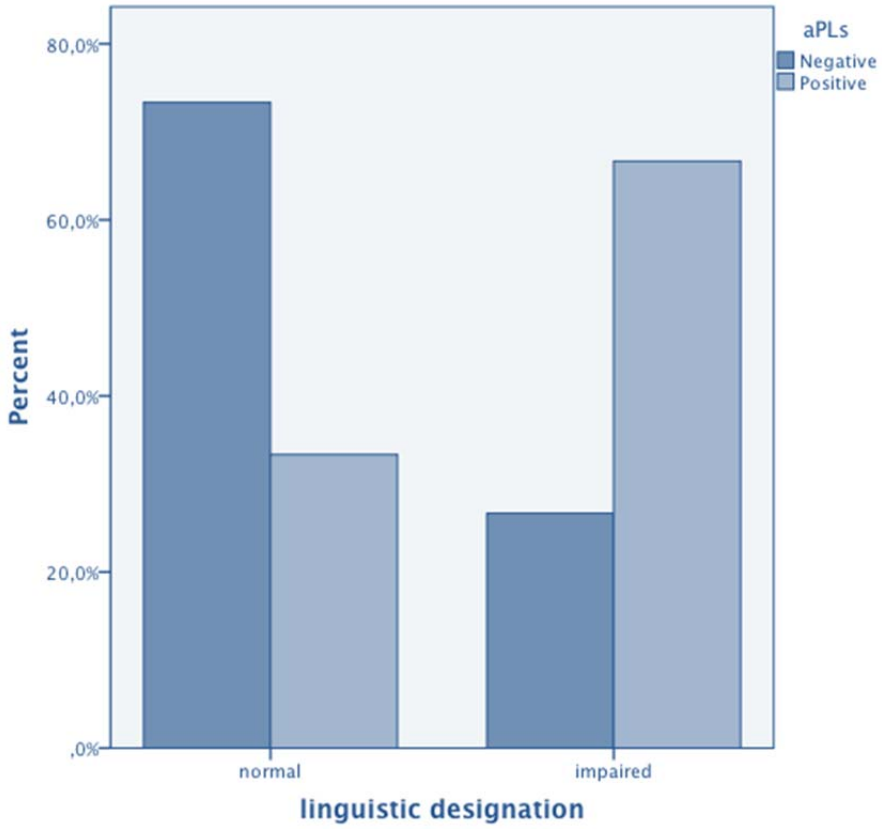
Statistical result

There was no association between CSF or serological aPLs positivity (s-aPLs) and depression or global cognitive impairment (p >0.05). When included serological B2GP1 limit values (5-9 units/ml) as aPLs positive instead of aPLs negative, we found an association between serological aPLs positivity and cognitive linguistic designation (p= 0.032) (Table 7) and (Figure 1).

Table 7. *Chi-Square tests show statistic association between serological aPLs positivity (including limit values for B2GP1) and cognitive linguistic designation impairment.*

	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,032	,020
Fisher's Exact Test	,032	,020

Figure 1.



Shows association between serological aPLs positivity (including limit values for B2GP1 in the positive group) and cognitive linguistic designation impairment expressed in percent.

Discussion

Several studies have proved connection between NPSLE manifestations and phospholipid antibodies in serum (56-58). Secondary APS due to SLE is common (41) and neuropsychiatric illness is common among APS patients as well (45). Today it's routine to test for anti-phospholipid antibodies in serum when sudden onset of cerebral symptoms in SLE patients occur. Mainly to rule out thrombotic disease, as an important piece in the NPSLE and APS diagnostic puzzles.

Aim 1

The results from previous studies testing anti-phospholipid antibodies in CSF are inconsistent. In our study we were not able to detect aCL or aB2GP1 IgG antibodies in CSF in any of our 51 subjects, although 14 of them were IgG aPLs positive in serum and 3 exhibited limit values for B2GP1. Our results are in accordance with result from a similar study performed by Jedryka-Goral et al. (59) Who also were unable to detect aCL in the CSF of NPSLE patients and healthy controls, even in sera aCL positive individuals. On the other hand, our results stand in contrast to results from Sokol et al. (32) and Martinez-Cordero et al. (60) Their results indicate that neuropsychiatric illness can cause intrathekal production of phospholipid antibodies, alternatively leakage of anti-phospholipid antibodies from systemic circulation to CSF, due to impaired blood brain barrier function. The diverse outcomes are probably due to the differences in study designs. The main differences are the composition of the study population, diagnostic criteria and inclusion/exclusions criteria. Most importantly is the different assay methods used to detect aPLs and its different limits of values that define (aPLs) positivity.

It's difficult to compare two similar groups, especially when the diagnosis is based on criteria as in SLE and NPSLE. The broad inclusion criteria that define disease positivity can give the study population a wide character. To improve our study design we need to include more patients, most importantly serological and CSF aPLs values from healthy controls and SLE patients without neuropsychiatric involvement. We also need to develop a normal range for aCL and aB2GP1 IgG antibodies in CSF and investigate if NPSLE patients CSF titers of these antibodies differences from CSF antibody titers in healthy controls and SLE patients without CNS involvement. Another improvement would be to collect and analyze CSF and serum samples for aPLs, IgM, IgG IgA at the same time and with the same analyze method. In our present study serum result was obtained by retrospective examination of medical files. This makes it impossible to determinate the type of analyze method used on the serological samples, as this is rarely noted. Different ELISA analyzes methods for detecting aCL and aB2GP1 are known to give varying results (61-64).

NPSLE are a relatively rare diagnose. Our study strength is the relatively large study population of 51 individuals, compared to other NPSLE studies, often investigating smaller populations. Another strength is the extensive screening of depression and cognitive impairment. The data collected can be of great value in future studies.

Aim 2

Cognitive impairment and depression is two of the most frequent reported neuropsychiatric syndromes in SLE patients (65, 66). Our extensive screening of depression and cognitive impairment confirms that these conditions are highly prevalent and often under diagnosed in NPSLE patients (table 6). The reasons behind these findings are harder to interpret. Both depression and cognitive impairment can be caused by primary NPSLE, APS or several non-NPSLE or APS related causes. Comorbidity between depression and cognitive impairment exists and these factors make it hard to evaluate the results separately and estimate the true disease incidence. Another concern to consider when testing cognitive function in SLE patients is that the symptoms can be fluctuating and hard to detect in one single test opportunity, one or more follow up tests is therefore recommended.

We found no statistic association between aPLs presence and depression. A higher percentage of aPLs negative NPSLE patients suffered from depression compared to aPLs positive NPSLE patients. There is data that suggest a possible connection between LA positivity and depression (33). Gao et al. (67) showed a correlation between depression like behavior in mice and titers of autoantibodies against DNA, NMDA receptors and aCL.

Serological presence of phospholipid antibodies has been shown to contribute to impaired cognitive function. Performances requiring speed and attention, concentration and visuospatial functions appear to be particularly affected (56, 68, 69). In our study we found a statistic association between cognitive linguistic designation impairment and aPLs positivity ($p<0.032$), although we only found significance when including limit values for β2GP1.

There are several possible cofounders not yet taken to consideration that may contribute development of cognitive impairment. It's possible that the prevalence of cognitive impairment increases with age. In our study the age of the population varied between 20-71 years, with a relatively low average age of 40 years, the average age was lower for aPLs positive 37,8 years respectively 41,8 years for aPLs negative. Another cofounder is the number of years with SLE diagnosis, as more years with SLE could increase the risk of cognitive impairment. Longer period of multi organ inflammation, accelerated arteriosclerosis and medical treatment, including prednisolone, increases the risk. Although some data suggest that it is uncertain if corticosteroid use contributes to development of cognitive impairment in SLE patients (70, 71). The aPLs positive average age with SLE diagnosis was 1,7 year longer than aPLs negative individuals (table 5) theses differences could have affected our statistical outcome. Other possible cofounders are disease activity level (SLEDAI) and chronic damage level (SLICC). In our groups there was no difference in average disease activity 8,6 for both groups and chronic damage levels was also similar 1,3 for aPLs positive respectively 1,4 for aPLs negative (table 5). All above-mentioned cofounders need to be taken into account for and investigated further, suggestively by using statistical regression analyses in future studies. One can also discuss if our limit value for cognitive impairment, (results below average age in one of six cognitive tests) is too low and needs to be elevated to two test results below average age, to enhance the security that it is true cognitive impairment that is exanimated (72). Further more, aPLs presence in patient's sera does not always cause clinical symptoms, including CNS involvement. We therefor need to consider the fact that the frequency of aPLs (1 -5%) among the healthy population without CNS involvement can affect our study outcome. It's therefore mandatory in future studies to screen the

study population and control groups for possible cofounders such as infections, neoplasm, active vaccination and drug therapies since they may affect and increase aPLs values (34).

Conclusions

How phospholipid antibodies gain access to CNS and affects neuropsychiatric functions remains a subject of investigation. It's unlikely to improve diagnosis of SLE patients with neuropsychiatric involvement, by testing CSF for cardiolipin and β2-glycoprotein1 IgG antibodies. Since all our CSF samples were negative, even when sera were positive. Although a larger study, in witch CSF and serological values are taken at the same time and in compression with a healthy controls and SLE patients without neuropsychiatric manifestations. As well as development of aCL and aβ2GP1 CSF normal range values, must be performed before we can draw this conclusion with absolute certainty.

Depression and cognitive impairment is common and often under diagnosed in NPSLE patients, regardless of aPLs positivity. Although an association between aPLs presence in serum and linguistic designation impairment is possible and needs to be exanimated further in future studies.

It is of great importance to continuously screen SLE patients with CNS involvement for depression and cognitive impairment in clinical practice. So that proper treatment can be initiated when needed and hopefully decrease morbidity and mortality in this patient group.

Populärvetenskaplig sammanfattning på svenska

Fosfolipidantikroppar i blod och ryggmärgsvätska, hos SLE-patienter med misstänkt CNS-engagemang och samband med depression och kognitiv nedsättning.

Autoimmunitet är när kroppens immunförsvar som är till för att bekämpa infektioner och döda eller skadade celler, börjar angripa den egna kroppens friska vävnader. Autoimmuna sjukdomar är vanligt inom reumatologin, en av dessa är Systemisk Lupus Erytematosus (SLE), en kronisk sjukdom som ofta drabbar unga kvinnor. Symptom från hjärnan och centrala nervsystemet är vanligt vid SLE och kallas då Neuropsykiatrisk SLE (NPSLE). Depression samt svårigheter att utföra uppgifter som kräver minne, inlärning, tidsuppfattning, beslutsfattande samt numerisk och språklig förmåga, s.k. kognitiv nedsättning är vanligt vid NPSLE och orsakar lidande och ökad dödlighet. Idag finns ingen enskild säker metod att diagnostisera NPSLE.

Vi undersökte förekomst av fosfolipidantikroppar i blod och ryggmärgsvätska hos 51 frivilliga SLE patienter med symptom från centrala nervsystemet, i hopp om att kunna förbättra diagnostiken av NPSLE. Patienterna undersöktes också för förekomst av depression och tecken på kognitiv nedsättning, för att se om det fanns ett möjligt samband mellan dessa tillstånd och förekomst av fosfolipidantikroppar i blod och/eller ryggmärgsvätska.

Ingen av patienterna visade förhöjda nivåer av fosfolipidantikroppar i ryggmärgsvätska, trotts att 14 av dem uppvisade förhöjda nivåer i blodet. Det gick

således inte att förbättra diagnostiken av NPSLE genom att testa ryggmärgsvätska för dessa antikroppar.

Vi fann däremot att depression och kognitiv nedsättning är vanligt och ofta underdiagnostiserat bland NPSLE patienter. Det är därför viktigt att kontinuerligt undersöka SLE patienter för dessa tillstånd så att rätt behandling kan sättas in i tid och minska lidande och dödligheten hos denna ofta unga patientgrupp. Ett visst samband mellan förekomst av fosfolipidantikroppar i blod och kognitiv nedsättning är möjligt, men vidare forskning krävs innan vi kan dra några säkra slutsatser.

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