Comparison of complement component levels in systemic lupus erythematosus and secondary antiphospholipid syndrome in regard to specific autoantibodies

Crnjak, Bernarda

UNIVERSITY OF SPLIT

SCHOOL OF MEDICINE

Bernarda Crnjak

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Diploma thesis

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LIST OF ABBREVIATIONS

ACR – American College of Rheumatology
ANA – Antinuclear Antibodies
aCL – Anticardiolipin Antibodies
aPL – Antiphospholipid Antibodies
APS – Antiphospholipid Syndrome
DMARDs – Disease-Modifying Antirheumatic Drugs
ELISA – Enzyme-linked Immunosorbent Assay
HLA-DR – Human Leukocyte Antigen–Antigen D Related
MAC – Membrane Attack Complex
MASP – Membrane-Associated Serine Protease
MBL – Mannose-Binding Lectin
MHC – Major Histocompatibility Complex
NSAIDs – Nonsteroidal Anti-Inflammatory Drugs
RI – Reference Interval
SLE – Systemic Lupus Erythematosus
SLICC – Systemic Lupus International Collaborating Clinic
TIA – Transient Ischemic Attack

1.INTRODUCTION

1.1. The Complement System

In 1896 the complement system was discovered and named after its objective to "complement" antibodies in their function to kill bacteria (1). The complement system is an essential part of the innate immune system and is composed of three independent but connected pathways that share a common purpose. They are called the classical, alternative, and mannose-binding lectin pathway. The complement system acts as a first line of defense to recognize and destroy pathogens and modified self-antigens, bridge the innate and adaptive immune systems, and eliminate immune complexes and products subsequent to inflammatory injury (2,3). The way in which the complement proteins help destroy pathogenic microorganisms and soluble antigens is by inducing an inflammatory response themselves (realized by anaphylatoxins, most notably C3a and C5a), thereby promoting chemotaxis, leukocyte activation and vasodilation. Complement components also serve as opsonins by marking antigens and thus enhance phagocytosis by phagocytic cells like macrophages. Terminal complement components also have the ability to directly kill unencapsulated, gram-negative pathogens by disrupting the cell membrane (1).

The complement system is composed of more than 30 plasma and membrane-associated proteins, and their function can be broken down into three units. The first unit represents proteins that activate the system, the second group of proteins has a regulatory function, and the last group acts as membrane-associated receptors that respond to complement components created during the activation. Complement proteins of the classical pathway are referred to by numbers (C1-9), of the alternative pathway by letter symbols (for example Factor H or D) and membrane-associated complement proteins often by trivial names (2). Predominantly produced by the liver, many complement plasma proteins are in an inactive state and only become active proteases after enzymatic cleavage (also called zymogens). Those proteases in turn exert their enzymatic activity on other zymogens down the complement pathway and thereby initiate a rapid complement response. The fragments of the cleaved proteins are designated with lowercase suffixes – for example C3 is cleaved into C3a and C3b (1,2).

As already mentioned there are three different proteolytic pathways known to activate complement. Even though they are distinctive in their mechanism of activation and target recognition, they ultimately converge at C3 which results in the formation of C3a, C3b, C5a and the membrane attack complex (C5b-9) (4) (Figure 1).

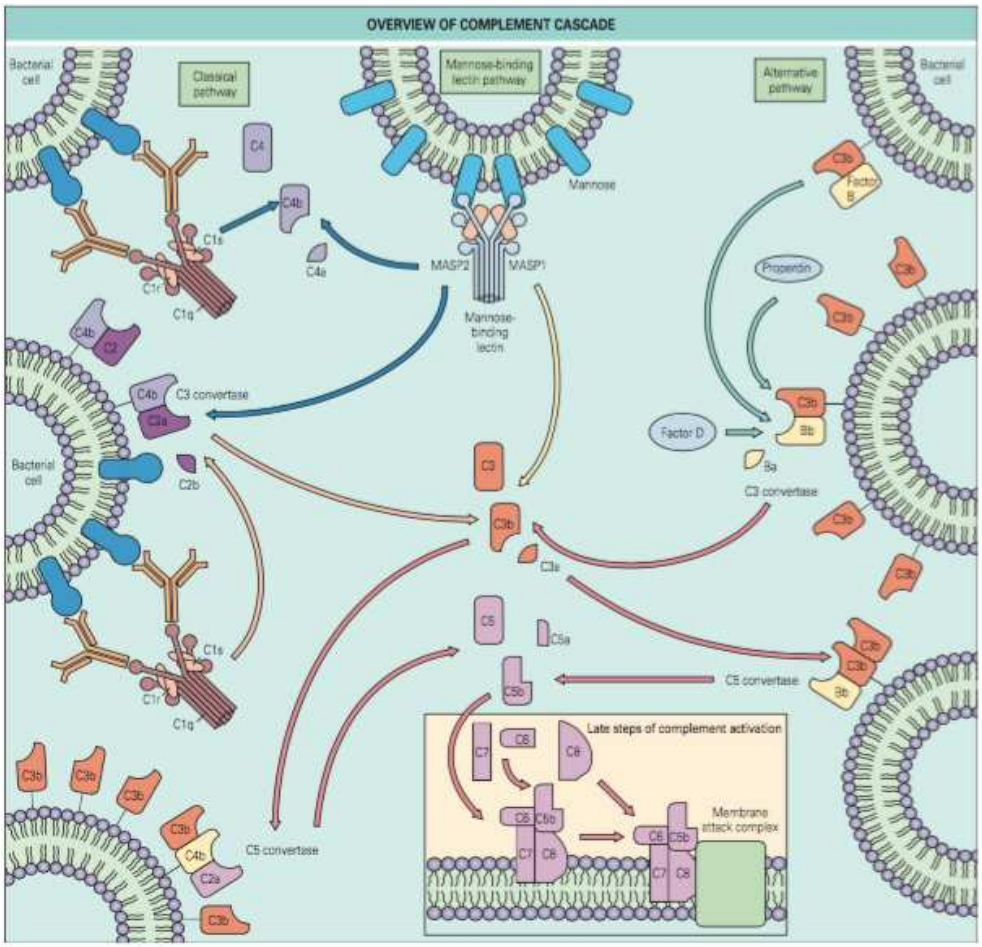

Figure 1. Overview of the complement cascade

Adapted from Hochberg M, Gravallese E, Silman AJ, Smolen J, Weinblatt M, Weisman M. Rheumatology. 7th ed. Philadelphia:Elsevier; 2007.

The classical pathway was the one being discovered first, but its activation is markedly dependent on a prior humoral immune response, and thus it is the last to have evolved. Immune complexes are formed when IgG or IgM immunoglobulins are complexed to pathogens or other non-self antigens. The multimeric C1 complex is made up of C1q, C1r and C1s molecules. The classical pathway is triggered, when the C1q binds to the Fc tails of the IgG or IgM immune complex. In response C1s and C1r go through a conformational change that activates and enables them to carry out their enzymatic activity. As a consequence, C1s then cleaves C4 and C2 to C4a and C4b as well as C2a and C2b, respectively. The assembly of C4b and C2a (C4b2a) generates the C3 convertase of the classical pathway, which enzymatically cleaves the central C3 component into C3a and C3b. This is the point where all three complement cascades converge. The C5 convertase is subsequently formed by the combination of C3b and C4b2a (C4b2a3b). At the same time C3b

also serves as an opsonin thus helping phagocytic cells. After C5 is cleaved into C5b and C5a, the MAC, that is made up of C5b, C6,C7,C8 and C9, is assembled. The MAC has the abilty to disrupt the cell membrane of pathogens which leads to pathogen cell lysis (1,4).

Unlike the classic pathway, the mannose-binding lectin pathway does not need a hummoral immune response to be initiated. The lectin pathway gets activated when mannose binding lectin or Ficolin bind to mannose or other carbohydrate residues, respectively. These carbohydrate residues are found on the surface of pathogens. MBL and Ficolin are associated with other MBL proteins (called MASPs) and exist as a MBL complex in the serum. After the complex encounters a mannose residue, there is a conformational change and subsequent autoactivation of MASP-1 and MASP-2. These continue to enzymatically cleave C4 and C2 which results in the assembly of C4b2a and thus formation of a C3 convertase analogous to the C3 convertase of the classical pathway (1,4).

The activation of the alternative pathway is not inititated by a pathogen-binding protein like the other pathways. It is continously activated by spontaneous hydrolysis of C3 in plasma, also called “tickover”, which produces C3b at a constant rate that is able to target bacteria. This hydrolysis can be intensified by contact of C3 with foreign and non-self antigens. Only C3b bound to a pathogen initiates binding of Factor B and this is ensued by binding of Factor D. Factor D enzymatically cleaves Factor B and C3bBb is formed, which is a functional equivalent to the C3 convertase of the classical pathway. A protein called properdin, that is made by activated neutrophils, attaches to the C3b part of the C3 convertase and stabilizes it. Recent studies have also found that properdin itself can bind to necrotic and apoptotic cells and thereby activate the complement cascade (1,4).

There is a connection between a dysregulated complement system and the pathogenesis of rheumatic diseases, most notably rheumatoid arthritis, SLE, APS, dermatomyositis, Sjögren syndrome, systemic sclerosis and vasculitides (Table 1). The mechanisms by which an aberrant complement response participates in the pathogenesis of these diseases are manifold. They result from complement component deficiency, overproduction, excessive activation of complement components, or inappropriate action of regulatory proteins. The link between autoimmune diseases and the complement system is somewhat contradictory: while its activation causes tissue damage in an ongoing disease, its deficiency at the same time renders someone more susceptible to the development of an autoimmune disease.

Table 1. Association between complement components and rheumatic diseases

Disease	Complement components associated with disease
Atypical hemolytic uremic syndrome (aHUS)	C3, factor H, MCP, factor I, Factor B, complement factor H-related proteins, Thrombomodulin
ANCA-associated vasculitis	Alternative pathway
Antiphospholipid syndrome	C3, C4, C5, C3a, C4a, MAC
C3 glomerulopathy and membranoproliferative glomerulonephritis (MPGN)	C3, alternative pathway
Sjögren syndrome	C4A, C4BP
Systemic lupus erythematosus (SLE) and lupus nephritis	C1q, C1r, C1s, C2, C3, C4, MBL, factor D

Adapted from Hochberg M, Gravallese E, Silman AJ, Smolen J, Weinblatt M, Weisman M. Rheumatology. 7th ed. Philadelphia:Elsevier; 2007.

In SLE for example, excessive complement activation leads to immune complex-induced inflammation and subsequent tissue damage. However individuals with hereditary complement deficiencies are more likely to develop SLE, for instance 93% of patients who have a C1q deficiency develop lupus (1). Individuals with hereditary C4 and C2 deficiencies are also more prone to develop SLE. Likewise acquired deficiencies like the production of autoantibodies against C1q as well as decreased levels of C3 and C4 can be noticed in SLE patients and are thought to participate in the development of the disease. C3 and C4 levels are even acknowledged means by which SLE disease activity is measured (also in the form of disease scoring systems, for example the systemic lupus erythematosus disease activity index incorporates hypocomplementemia as a laboratory variable) (5). The lack of key complement components leads to defective clearance of immune complexes that subsequently deposit in multiple organs and vessels, thereby causing tissue damage and vasculitis. The absence of certain complement components also causes defective clearance of apoptotic cells and abnormal recognition of self-antigens by B cells (loss of immune tolerance), which eventually leads to the development of autoimmunity (4).

Recent in vivo studies have shown that the activation of the complement cascade, especially the classical pathway, is an essential mediator of pregnancy morbidity and the thrombogenic effect of aPL antibodies, and that C3a, C5a and C5b-9 MAC, are key components of this process (7). Studies have also shown that due to complement activation, patients with primary APS present with hypocomplementemia (decreased C3 and C4 and increased C3a and C4a levels) (6). Therefore, complement components may serve as targets and be of great significance in the light of novel therapy approaches (7,8).

1.2. Rheumatologic autoimmune diseases: Systemic Lupus Erythematosus and Antiphospholipid Syndrome

Autoimmune disease describes the process of the immune system attacking self-antigens secondary to the loss of immunologic tolerance (9). The immunologist Paul Ehrlich used the term "horror autotoxicus" in the 20th century to illustrate how catastrophic the idea of the body's immune system turning against his own tissues was, back then thinking that it was not compatible with life (10). Present-day believes suggest that an interplay of genetic susceptibility as well as environmental factors contribute to the dysregulation of the immune system that ultimately leads to tissue destruction (11). There is a differentiation between the physiologic self-reactivity necessary for maintaining healthy immune system homeostasis that can protect against infection and the pathologic self-reactivity that is commonly known to result in a clinically manifested autoimmune disease. They can be classified as systemic, one of them being Systemic Lupus Erythematosus, or organ-specific, one of them being Antiphospholipid Syndrome. Systemic disease is characterized by the immune system attacking ubiquitously expressed self-antigens, while in organ specific disease the self-antigens are limited to a specific cell or tissue type. In both, autoantibodies, T cells and various other immune cells are the culprit of end-organ damage with pathophysiologies being very diverse amongst different diseases (12).

SLE causes the production of autoantibodies to nuclear antigens (ANA) and a very broad clinical picture that ranges from milder manifestations like skin rashes and non-erosive arthritis to severe complications such as lupus nephritis or neuropsychiatric disorders. The principal pathologies are inflammation, vasculitis, immune complex and subsequent complement deposition, and vasculopathy, the latter especially occurring in the presence of antiphospholipid antibodies (13).

APS is characterized by a hypercoagulable state with repeated venous, arterial and small-vessel thrombosis as well as pregnancy complications in the presence of antiphospholipid antibodies (14). It may exist as an isolated disease (primary APS) or in association with other rheumatic diseases, primarily SLE, also known as secondary APS (12). It is estimated that 30-40% of SLE patients are positive for antiphospholipid antibodies (15).

1.2.1. **Systemic lupus erythematosus**, the "disease with a thousand faces" (16), is a chronic multisystem autoimmune inflammatory disease that clinically presents with wide-ranging manifestations. Biochemically it is characterized by immune complex formation and ANA as well as anti-double stranded DNA antibodies. For the diagnosis of systemic lupus erythematosus these antibodies have the greatest sensitivity and specificity, respectively (12).

The reported incidence and prevalence of systemic lupus erythematosus varies greatly which is due to different populations being studied as well as different methodological approaches used amongst studies for identifying cases of SLE. Estimated prevalence varies from 20 to 240 per 100,000 persons, while incidence varies from 1 to 10 per 100,000 person-years (17). Most commonly affected populations are women of childbearing age (15-45 years) as well as populations of nonwhite ethnicity and race like African-Americans, Asians, and Hispanics (12).

The definite mechanisms that lead to the development of SLE continue to be very elusive. It is most likely attributable to an interplay of genetic variations and environmental triggers that make a person more susceptible to the development of this disease. Nowadays it is established that disease-associated genetic susceptibility affects almost all components of both the innate and adaptive immune system, which in turn are responsible for the development of SLE and also for the subsequent susceptibility to environmental factors (12). Some of these components are the loss of immunotolerance and a high antigenic load. Likewise the excess of type 2 T helper cells and insufficient B cell suppression are responsible for the B cell overactivity and the generation of autoantibodies with subsequent immune complex formation (13). Research has also shown that variants in the HLA region on chromosome 6 encoding glycoproteins, known as MHC, makes people more susceptible to the development of SLE. Particularly the HLA-DRB1 in the class II region shows a strong association. These glycoproteins play a major role in antigen presentation. The complement system assumes a two-faced role in the pathogenesis of SLE. Development of SLE displays an association with genetic deficiencies of early complement pathway components. The strongest association can be found with homozygous deficiencies of the components C1q, C4

and C2 with subsequent development of SLE in >90%, 50% and 10-20% of cases, respectively. At the same time paradoxically the complement system, especially the classical pathway, is being activated in patients with SLE and represents a cause of tissue damage. Therefore complement component levels are also used to measure disease activity (1,18).

As mentioned before, environmental influences and certain risk factors also play a role in triggering SLE apart from the genetic predisposition. The female to male ratio of 9:1 found in the childbearing period already points to hormonal and reproductive contributions as risk factors. Studies have shown that lupus patients had significantly higher levels of estradiol and prolactin in addition to lower levels of androgens (19). From all the environmental triggers linked to SLE, exposure to ultraviolet light has a definite role in the pathogenesis. UV light can lead to flares with systemic symptoms and signs of the disease (20). Also, a positive relation between oral contraceptive use, postmenopausal hormone replacement therapy and SLE incidence has been established (21). There might also be a connection to past Epstein-Barr Virus infection since SLE patients show significantly higher seropositivity rates (22). The risk of developing SLE is also significantly increased in current smokers (23), people being treated with lupus-inducing medication like hydralazine or procainamide (24) and with exposure to crystalline silica (25).

The clinical picture of SLE is very heterogenous as is the clinical course with periods of remissions and relapses. The disease may involve virtually any organ system in the body. The most common symptoms are constitutional with pronounced fatigue, fever, loss of appetite and weight, and malaise (Table 2). Almost just as often patients complain of musculoskeletal symptoms. Particularly arthtralgias most commonly manifest as the presenting symptom of the disease. Arthritis and joint pain usually affect small joints of the hand, wrist and knee. Other symptoms are non erosive arthritis, avascular osteonecrosis, myalgias and myositis. Also worth mentioning is iatrogenic osteoporosis and subsequent fractures due to longstanding glucocorticoid therapy (1,26).

Table 2. Approximate prevalence of selected symptoms, signs, and laboratory abnormalities in SLE

Symptoms, sign, or laboratory abnormality	Prevalence (%)
Positive antinuclear antibody	97
Malaise and fatigue	90
Arthralgia, myalgia	90
Sun sensitivity, skin changes	70
Cognitive dysfunction	70
Low C3 or C4 complement	61
Fever caused by lupus	57
Antibodies to ds DNA	50
Arthritis	50
Leukopenia	46
Pleuritis	44
Anemia	42
Alopecia	40
Nephritis, proteinuria	40
Anticardiolipin antibody	35
Malar rash	35
CNS	32
Increased gamma globulin	32
Weight loss caused by lupus	27
Raynaud phenomenon	25
Hypertension	25
Sjögren syndrome	25
Oral ulcerations (mouth, nose)	20
Myositis	10
Avascular necrosis	10

Adapted from Hochberg M, Gravallese E, Silman AJ, Smolen J, Weinblatt M, Weisman M. Rheumatology. 7th ed. Philadelphia:Elsevier; 2007.

Lupus was historically described as a dermatologic disease and correspondingly has prominent dermatologic manifestations (26) (Table 3). Dermatologic features specific to lupus can be acute, subacute or chronic discoid. Even though only seen in 30% of pateints, the best-known acute dermatologic feature is the malar/butterfly rash with or without a disseminated maculopapular eruption. Subacute cutaneous lupus erythematosus usually presents as scaling papules/plaques that are diffuse and nonscarring. They resemble psoriasis or lichen planus and are associated with autoantibodies to Ro in up to 90% of cases. Other manifestations are photosensitivity to ultraviolet radiation, alopecia, mucous membrane lesions, oral and nasopharyngeal ulcerations, and discoid lesions. The latter can be part of chronic discoid SLE as well as the main pathologic feature in a separate entity called Discoid

Lupus Erythematosus, a disease associated with scarring and atrophy exclusively limited to the skin without organ involvement. Raynaud phenomenon, periorbital edema, livedo reticularis (often associated with elevated aPL or severe vasculitis), panniculits, bullous lesions, vasculitic purpura, telangiectasias, and urticaria are other dermatologic manifestations found in, but not unique to, SLE (1,26).

Table 3. Skin lesion per modified Gilliam classification – Cutaneous manifestations of SLE

Types of cutaneous lupus
Acute cutaneous lupus erythematosus
Subacute cutaneous lupus erythematosus
Chronic (discoid) lupus erythematosus
Seen in <1% of cases: hypertrophic lupus, lupus tumidus, lupus profundus, lupus pernio (chilblains), bullous lupus
Cutaneous manifestations of systemic lupus erythematosus
Sun sensitivity
Oral, nasal, or genital ulcerations
Malar rash
Hair loss or thinning
Changes in pigmentation
Urticaria
Calcinosis
Telangiectasias
Cutaneo-vascular manifestations of systemic lupus erythematosus
Cutaneo-vascular manifestations of lupus
Cryoglobulinemic vasculitis
Raynaud phenomenon
Livedo reticularis
Erythromelalgias
Ulceration or gangrene
Purpura

Adapted from Hochberg M, Gravallese E, Silman AJ, Smolen J, Weinblatt M, Weisman M. Rheumatology. 7th ed. Philadelphia:Elsevier; 2007.

One of the most debilitating manifestations is renal involvement, with renal failure being a common cause of morbidity and mortality in SLE patients. While almost all patients (90%) have renal biopsies that show immune complex and complememt depositions, only about 50% of the patients will develop clinically manifest lupus nephritis. Since renal dysfunction usually starts within the first 3 years of disease onset, regular screening is vital during this period. Aside from the immune complex-mediated glomerulonephritis with inflammatory cell infiltration that the vast majority of patients presents with, renal

involvement can also manifest als tubulointerstital or vascular disease (1). Often tubulointerstital or vascular disease is co-occuring with glomerulonephritis and even up to 66% of patients have tubulointerstital disease on the renal biopsy specimen (12). Possible symptoms of lupus nephritis are hematuria, hypertension, proteinuria (more than 0.5g/day) with corresponding low serum albumin and edema, and uremia. Prognosis and exact management are based on the extent, activity and pathologic classification of renal disease (1).

Other manifestations of SLE can be neuropsychiatric (headache, psychiatric disorders, cognitive dysfunction), gastrointestinal (acute abdominal pain, anorexia, nausea), lympathic and hematologic (lymphadenopathy, anemia, leukopenia, thrombocytopenia), cardiopulmonary (pleuritic discomfort, myocardial dysfunction, pleural effusion), accelerated atherosclerosis (coronary artery disease, cerebral vascular disease, peripheral vascular disease), and eyes, ear, nose and throat involvement (keratoconjunctivitis sicca, retinal vascular changes, sudden-onset sensorineuronal hearing loss). Aside from the fact that they show a greater incidence of hematologic malignancies, patients with SLE are also very susceptible to infections due to disease-related and therapeutic reasons (1).

Laboratory findings in patients with SLE are a high erythrocyte sedimanetation rate, normal or only slightly elevated C-reactive protein, cytopenias or other hematologic abnormalities like autoimmune hemolytic anemia. Antinuclear antibodies should be assessed by indirect immunofluorescence tests and additional differentiaion of antinuclear antibodies should take place in the setting of a positive ANA titer (anti-Sm, anti-double-stranded DNA, anti-ribosome P, anti-proliferating cell nuclear antigen, anti RNA helicase A, anti-Ro/SSA, -La/SSB autoantibodies, etc.). Further laboratory tests should include the complement levels of C3 and C4, which are especially lowered in active disease, antiphospholipid antibodies and lupus anticoagulant so as to assess possible APS (1). To determine the extent of renal involvement serum creatinine and glomerular filtration rate should be measured, urinary status and sediment examined, and 24-hour urine collected (1,27). Further tests depend on the specific symptoms a patient experiences and should be individually adjusted.

Since SLE is a multisystem disease, it's diagnosis can be rather difficult. Therefore, the diagnosis is mostly established with the help of an experienced rheumatologist on the basis of the clinical picture as well as serologic or immunologic testing. Recognizing and integrating preclinical aspects like the production of autoantibodies, immune complex formation or demographic features can help recognize patients that are still asymptomatic or symptomatic but do not meet enough criteria to be classified as SLE.

Criterion	ACR 1971	ACR 1982	ACR 1997	SLICC 2012
Malar rash	Malar rash	Malar rash	Malar rash	Acute cutaneous rash
Discoid rash	Discoid rash	Discoid rash	Discoid rash	Chronic cutaneous rash
Raynaud phenomenon	Raynaud phenomenon			
Alopecia	Alopecia			Nonscarring alopecia
Photosensitivity	Photosensitivity	Photosensitivity	Photosensitivity	
Oral or nasal ulcers	Oral or nasal ulcers	Oral or nasal ulcers	Oral or nasal ulcers	Oral or nasal ulcers
Arthritis	Arthritis without deformity	Nonerosive arthritis in at least two peripheral joints	Nonerosive arthritis in at least two peripheral joints	Synovitis involving at least two joints *or* tenderness in at least two joints with at least 30 min of morning stiffness
Serositis	A. Pleurisy B. Pericarditis	A. Pleurisy B. Pericarditis	A. Pleurisy B. Pericarditis	A. Pleurisy B. Pericarditis
Renal disorder	A. Profuse proteinuria B. Cellular casts	A. Profuse proteinuria B. Cellular casts	A. Profuse proteinuria B. Cellular casts	A. Profuse proteinuria B. Red blood cell casts
Neurologic	A. Psychosis B. Convulsions	A. Psychosis B. Seizures	A. Psychosis B. Seizures	A. Psychosis B. Seizures C. Mononeuritis multiplex D. Myelitis E. Peripheral or cranial neuropathy F. Acute confusional state
Hematologic	A. Hemolytic anemia B. Leukopenia C.Thrombocytopenia	A. Hemolytic anemia B. Leukopenia C.Thrombocytopenia D. Lymphopenia	A. Hemolytic anemia B. Leukopenia C.Thrombocytopenia D. Lymphopenia	A. Hemolytic anemia B. Leukopenia C. Lymphopenia D. Thrombocytopenia
Immunologic	A. LE cells B. False-positive STS	A. LE cells B. False-positive STS C. Anti-DNA D. Anti-Sm	A. False-positive STS B. Anti-DNA C. Anti-Sm D. APS E. Lupus anticoagulant	A. Anti-DNA B. Anti-Sm C. APS C1. Lupus anticoagulant C2. False-positive rapid plasma reagin C3. At least medium anticardiolipin ab C4. Anti–β2-glycoprotein I D. Low complement (C3, C4, CH50) E. Direct Coombs test in absence of hemolytic anemia
Antinuclear antibody	Positive ANA	Positive ANA	Positive ANA	Positive ANA

Figure 2. Evolution of criteria classification systems for SLE

Adapted from Hochberg M, Gravallese E, Silman AJ, Smolen J, Weinblatt M, Weisman M. Rheumatology. 7th ed. Philadelphia:Elsevier; 2007.

To help doctors diagnose patients suffering from SLE and to more easily identify patients for clinical trials the American College of Rheumatology has released guidelines in 1971 with revisions in 1982 and 1997 (Figure 2). Consistent with these guidelines a patient is classified as having SLE if 4 out of 11 criteria are met and differential diagnoses have been excluded. In 2012, the SLICC have revised the ACR guidelines and determined 11 clinical and 6 immunologic criteria to classify a patient as having SLE (Figure 2). According to the SLICC guidelines a patient is diagnosed with SLE if he has either a positive anti-nuclear or anti-double stranded DNA antibody titer in addition to lupus nephritis proven with a renal biopsy or if he fulfills 4 of the criteria with at least one being immunological and one clinical. SLICC guidelines' additional criteria are a positive direct Coombs test without having hemolytic anemia, lowered complement levels as well as further neurologic and dermatologic aspects. Both guidelines are in use and diagnosis can also be made by an experienced clinician without the need of fulfilling all the criteria postulated (1).

There are several phenotypic subgroups of SLE that can clinically overlap and are not mututally exclusive. They are classified according to the coexistence of a certain clinical picture and specific autoantibodies: anti-dsDNA antibodies with glomerulonephritis; anti-Ro/SSA antibodies with SLE, subacute cutaneous lupus, neonatal lupus, Sjögren syndrome and primary biliary chirrhosis; antiphospholipid antibodies with SLE accompanied by vasculopathy, misscarriages, livedo reticularis and stroke (secondary APS). Lastly there is the syndrome of SLE-mixed connective tissue disease overlap with anti-U1 ribonucleoprotein antibodies, arthritis, Raynaud phenomenon, myositis as well as pulmonary hypertenesion (1).

Treatment is based on anti-inflammatory and immunosuppressive agents, DMARDs and organ specific treatment. The mainstay of treatment for osteoarticular symptoms are NSAIDs that may be combined with hydroxychloroquine, an antimalarial drug belonging to the DMARDs. If the response to this treatment is unsatisfying, other DMARDs like methotrexate are used (1). For dermatologic manifestations a combination of nonpharmacologic and pharmacologic treatment is needed. There is a general advice to use sun protection, avoid photosensitizing drugs and stop smoking. Pharmacologic therapy is based on topical glucocorticoids and calcineurin inhibitors, systemic antimalarial drugs and glucocorticoids (1). First-line treatment for severe cytopenias and cardiopulmonary disease are glucocorticoids. Treatment of neuropsychiatric manifestations depends on the presentation and can range from supportive/symptomatic therapy with anxiolytics or anticonvulsants, immunosuppressive therapy with corticosteroids, azathioprine, or cyclophosphamide to anticoagulation with warfarin in case of vascular disease (1). Renal involvement presents itself in diverse clinical pictures and thus treatment is individually tailored to every patient based on blood pressure measurement, the presence of dyslipidemia as well as specific investigations like urinalysis, renal biopsy, serologic testing and daily protein excretion. There is an induction and maintenance therapy with a combination of immunosuppressive agents and DMARDs like glucocorticoids, mycophenolic acid, cyclophosphamide, azathioprine and various other agents. Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers are the mainstay of treatment for hypertension while statins are used for dyslipidemias (1). In case of positive antiphospholipid antibodies, estrogen-containing hormonal contraceptives should be avoided and antiplatelet-anticoagulant therapy may be added to overcome the thrombotic diathesis (28,29).

1.2.2. **Antiphospholipid Syndrome** is an autoimmune disease associated with arterial and venous thrombosis, pregnancy morbidity (fetal loss, premature birth, miscarriage), and the presence of a miscellaneous group of autoantibodies called antiphospholipid antibodies. The aPLs are lupus anticoagulant, anticardiolipin antibodies, and anti-β2 glycoprotein-I antibodies. They mostly target phospholipid-binding proteins instead of phospholipids themselves. If there is no other underlying autoimmune disease, it is referred to as "primary" APS, while in the presence of SLE or any other autoimmune disease it is denoted "secondary" APS (1).

Even though the prevalence of antiphospholipid antibodies in the general population is reported to be somewhere between 1% to 5%, depending on the population studied, only a small fraction of those individuals will experience APS (1,30). A couple of epidemiological studies suggest that the incidence is around 5 new cases per 100.000 persons per year. Prevalence increases with age and in individuals with a chronic disease and is estimated to be 40 to 50 cases per 100.000 persons (30). Primary APS is responsible for approximately 15% of cases of deep vein thrombosis with or without consecutive pulmonary embolism, one third of new onset strokes in individuals younger than 50 years, and 10% to 15% of recurrent fetal death during pregnancy. Patients with SLE and secondary APS syndrome also suffer from thromboembolic events and recurrent pregnancy loss. Antiphospholipid antibodies are found in 30% to 40% of patients suffering from SLE, while 10% to 15% of SLE patients show clinically significant symptoms of APS. In families with APS a genetic predisposition is the probable foundation for disease development and a relationship with human leukocyte antigen DR7, DR4, C4 null allele and other gene complexes has been established (1).

Pathogenesis of APS is not completely understood and the heterogenous nature of complications suggests that more than one pathological mechanism contributes to the development of the disease (31). Thrombosis is amongst other things due to the interaction of aPLs with monocytes, thrombocytes and endothelial cells. The interaction results in a prothrombotic phenotype. Since aPLs ("first hit") in the serum are continuously present and thrombosis only comes about occasionally, it was hypothesized that another additional thrombophilic event ("second hit") has to occur. Other mechanisms that lead to thrombosis are complement activation by aPLs with excess C3a and C5a generation, interference with clotting regulatory proteins, and increased release of neutrophil extracellular traps (1). The exact pathophysiology of pregnancy loss is still unknown. One the hand thrombosis seems to be a definite mechanism since observational studies have shown placental thrombosis and infarction as the etiology of pregnancy loss. On the other hand, not all patients with obstetric

APS have placental infarction or vasculopathy and other mechanisms have been suggested by in vitro studies. aPLs may interfere with implantation of the trophoblast into the maternal uterus and lead to decreased production of human chorionic gonadotropin. Activation of the classical pathway of the complement cascade by aPLs was shown to be a causative mechanism of pregnancy morbidity since the formed anaphylatoxins (C3a, C5a) promote tissue injury in addition to a prothrombotic profile. Another causative process is that anti-β2 glycoprotein-I antibodies seem to be implicated in defective placentation and the displacement of annexin V, which usually has an anticoagulant function for the trophoblast (1,31).

Apart from thromboses and pregnancy morbidity, the clinical picture of APS can also present itself with only thrombocytopenia or hemolytic anemia. Any organ can be diseased and thus the set of clinical features is extremely diverse. Primary APS can evolve into secondary APS with SLE, and some patients that initially present with only SLE go on to develop secondary APS (1).

Venous thrombosis most commonly affects the deep veins of the lower extremities and arterial thrombosis most often presents itself as an ischemic stroke or transient ischemic attack, although occlusion can happen in any part of the vascular tree like the retinal, mesenteric, or peripheral arteries. In the general population fetal loss usually occurs in the first trimester as opposed to APS pregnancy morbidity that most commonly manifests itself in the second and third trimesters. Apart from fetal loss and distress and premature birth, pregnant women may also experience complications like preeclampsia, intrauterine growth restriction, premature delivery and others. Cardiac valve abnormalities are a highly prevalent feature of APS. Usually it is the mitral valve that shows thickening, nodules and vegetations. The abnormalities may lead to stenosis, regurgitation or even emboli causing stroke and TIAs, but may also be clinically nonsignificant. Even though there is no pathognomonic skin lesion in APS the skin feature most commonly encountered is livedo reticularis. Some of the other manifestations are leg ulcers, superficial thrombophlebitis, cutaneous gangrene (usually due to occlusion of small arteries), and gangrene of the extremities. Thrombocytopenia, mostly in the range of 100000 to 150000 per mm^3, is often seen in APS patients and usually does not cause hemorrhage. Hemolytic anemia seldom occurs, even though 10% to 20% of the patients have a positive Coombs test. Renal involvement in the form of thrombotic microangiopathy can lead to renal failure with hypertension and proteinuria (1,31).

Catastrophic APS is variant of APS and can lead to multiorgan failure and be potentially fatal. It is characterized by positive serology for aPLs and the sudden and simultaneous (or within less than a week) start of microvascular thrombosis in at least 3 organ sites (1,12). It has been proposed that extensive complement activation contributes to the pathogenesis and development of catastrophic APS. This theory is supported by case reports of patients that have been successfully treated with eculizumab, a C5 inhibitor, and at the same time not being responsive to treatment with anticoagulants (31).

Diagnosis is based on the Sapporo Criteria that were established in 1966 and revised in Sydney 2006 (Figure 3). A patient needs to meet at least one clinical and one laboratory criteria to be diagnosed with APS. Clinical criteria are vascular thrombosis and pregnancy morbidity, while laboratory criteria are the presence of aPLs measured by a standardized ELISA at least 12 weeks apart.

Clinical Criteria
1.Vascular thrombosis One or more clinical episodes of arterial, venous, or small vessel thrombosis in any tissue or organ 2. Pregnancy morbidity (a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, or (b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of eclampsia, severe pre-eclampsia, or recognized features of placental insufficiency, or c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded
Laboratory Criteria
1. Lupus anticoagulant present in plasma on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis 2. Anti-cardiolipin antibody of IgG or IgM isotype in serum or plasma, present in medium or high titer (>40 IgG phospholipid or IgM phospholipid, or >99th percentile), on two or more occasions at least 12 weeks apart, measured by a standardized ELISA 3. Anti–β-2-glycoprotein I antibody of IgG or IgM isotype in serum or plasma (in titer >99th percentile) present on two or more occasions at least 12 weeks apart, measured by a standardized ELISA
Definite APS is present if at least one of the clinical criteria and one of the laboratory criteria are met. Classification of APS should be avoided if less than 12 weeks or more than 5 years separate the positive anti-phospholipid antibody test and the clinical manifestation.

Figure 3. Revised Sapporo Classification Criteria for APS

Adapted from Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR. Kelley and Firestein's Textbook of Rheumatology. 10th ed. Philadelphia: Elsevier; 2017.

Treatment of APS depends on the clinical picture. aPL positive people that are asymptomatic don't receive treatment. Patients with an acute episode of thrombosis receive heparin, while patients with a past thrombotic event receive warfarin for secondary prophylaxis. Warfarin can be combined with aspirin in case of recurrent thrombosis. Prgenant women that are aPL positive and had a previous fetal loss are treated with unfractioned or low-molecular-weight heparin, that can be supplement by low-dose aspirin. Thrombocytopenia with a count of less than 50,000/mm^3 is treated with prednisone and intravenous immunoglobulin. Adequate therapy of catastrophic APS consists of anticoagulation, corticosteroids, intravenous immunoglobulin, and plasmapheresis. If there is no improvement cyclophosphamide, rituximab, or eculizumab, the latter being a terminal complement inhibitor, have been proposed as an alternative treatment option (12).

2. OBJECTIVES

The purpose of the study is to compare complement component levels amongst two groups of patients, one group of patients having SLE as a diagnosis, while the other group has SLE and secondary APS as a diagnosis. Also, the correlation between complement component levels and anti-dsDNA and anticardiolipin autoantibodies has been determined, respectively.

Hypothesis

1. Complement component levels in patients with SLE and secondary APS are significantly lower compared to patients with SLE without secondary APS.
2. Complement component levels are related to ds-DNA and anticardiolipin antibodies.

3. MATERIALS AND METHODS

3.1. Study Design

This cross-sectional study was conducted in the Department of Immunology and Rheumatology of the University Hospital Split (KBC Split) of the University of Split, School of Medicine and data were collected from the period of January 2006 to May 2018.

3.2. Study Population

In this study 74 patients were included, from which 45 were diagnosed with SLE and 29 were diagnosed with SLE and secondary APS. All patients were diagnosed by a rheumatologist on the basis of the ACR criteria from 1997 for the classification of SLE and on the basis of revised Sapporo Criteria from 2006 for the Classification of APS. Exclusion criteria was the diagnosis of any other coexisting rheumatologic disease apart from SLE or SLE with secondary APS, respectively. Eligible patients were identified using the database at the Department of Immunology and Rheumatology of the University Hospital Split (KBC Split).

3.3. Materials

Medical data of eligible patients were retrieved from the Institute of Laboratory Diagnostics at the University Hospital Split, location Križine. Following laboratory data were collected for each patient, if available:

1. ANA antinuclear antibodies (positive/negative)
2. anti-ds DNA antibodies (negative if <30)
3. C3 (RI 0.9-1.8)
4. C4 (RI 0.1-0.4)
5. IgG (RI 6.5-16.0)
6. IgM (RI 0.5-3.0)
7. IgA (RI 0.4-3.5)
8. Lupus Anticoagulant (negative if <1.20)
9. IgG/IgM anticardiolipin antibodies (negative if <15/12.5)
10. IgG/IgM beta2-gylcoprotein 1 antibodies (positive if >20)

3.4. Statistical Evaluation

Data analysis was conducted using the statistical software STATISTICA 12 (TIBCO Software Inc. v12.0). In this study data has been reproduced in the form of tables and graphs. The normal distribution of data has been tested with the Kolmogorov-Smirnov Test. To determine the difference and the significance for numeric and normally distributed variables between the two studied groups, the T-test has been used. Non-numeric variables were tested with Chi-Square test or alternatively with Fisher exact test. The significance level was determined to be $P < 0.05$.

4. RESULTS

The 45 patients included in the study and diagnosed with SLE have a lowered C3 complement level that averaged 0.88 (reference interval of 0.9-1.8), with a standard deviation of 0.26 and a 95% confidence interval that defines a range of 0,8 to 0,96. In contrast, the C4 complement level lies within the reference interval (0.10-0.40), nevertheless it is determined to be at the lower range of normal with a mean of 0.14, a standard deviation of 0.08, and a 95% confidence interval that defines a range of 0.11 to 0.16.

The IgG values, only retrieved in 44 participants, were within the reference interval (6.5-16.0) with a mean of 11.87 and a standard deviation of 4.44. (Table 4)

Table 4. Descriptive Statistics of C3, C4 and IgG for patients with SLE

Category = SLE Descriptive Statistics					
	Valid N	**Mean**	**Confidence - -95.000%**	**Confidence - 95.000%**	**Std.Dev.**
C3	45	0.88	0.80	0.96	0.26
C4	45	0.14	0.11	0.16	0.08
IgG	44	11.87	10.52	13.21	4.44

Patients that are diagnosed with SLE and secondary APS have a C3 complement level that lies within the reference interval (0.9-1.8), with a mean of 0.90, and a standard deviation of 0.23 as well as a calculated 95% CI of 0.82 to 0.99. Likewise, the C4 complement level lies within the reference interval with a mean of 0.12 (RI 0.10-0.40), a standard deviation of 0.07, and a 95% CI of 0.10-0.15. Both complement component levels were in fact within the RI, but it bears mentioning that both variables were found at the lower border of normal.

The IgG values, reported in 27 participants, were within the reference interval (6.5-16.0) with a mean of 14.76 and a standard deviation of 17.55. (Table 5)

Table 5. Descriptive Statistics of C3, C4 and IgG for patients with SLE and secondary APS

Category = SLE + APS syndrome Descriptive Statistics					
	Valid N	**Mean**	**Confidence - -95.000%**	**Confidence - 95.000%**	**Std.Dev.**
C3	29	0.90	0.82	0.99	0.23
C4	29	0.12	0.10	0.15	0.07
IgG	27	14.76	7.82	21.71	17.55

The variables gender and diagnosis are not independent. There is a statistically significant preponderance of the female gender in both groups (*P* value = 0.016). (Table 6)

Table 6. Observed gender frequencies

2-Way Summary Table: Observed Frequencies			
	Gender - Female	**Gender - Male**	**Row - Totals**
SLE + APS syndrome	22	7	29
%	75.86%	24.14%	
SLE	43	2	45
%	95.56%	4.44%	
Totals	65	9	74
Fisher exact test (1-side)			0,0158

In both groups IgG aCL antibody is mostly negative. The Chi-Square test didn't prove that IgG aCL antibody is more specific for any patient group (*P* = 0.217). (Table 7)

Table 7. Observed IgG aCL antibody frequencies

2-Way Summary Table: Observed Frequencies			
	IgG aCL Antibody - negative <15	**IgG aCL Antibody - positive**	**Row - Totals**
SLE	30	11	41
%	73.17%	26.83%	
SLE + APS syndrome	14	10	24
%	58.33%	41.67%	
Totals	44	21	65
Chi-Square test			0,2170

In both groups IgM aCL antibody is mostly negative. The Chi-Square test didn't prove that IgM aCL antibody is more specific for any group of patients ($P = 0.863$). (Table 8)

Table 8. Observed IgM aCL antibody frequencies

2-Way Summary Table: Observed Frequencies			
	IgM aCL Antibody - negative < 12.5	**IgM aCL Antibody - positive**	**Row - Totals**
SLE	28	12	40
%	70.00%	30.00%	
SLE + APS syndrome	18	7	25
%	72.00%	28.00%	
Totals	46	19	65
Chi-Square test			0.863

In both groups IgG beta2-glycoprotein 1 Antibody is mostly negative. The fisher exact test proves that a negative IgG beta2-glycoprotein 1 Antibody is more specific for the patient group diagnosed with SLE ($P = 0.011$). (Table 9)

Table 9. Observed IgG beta2-glycoprotein 1 antibody frequencies

2-Way Summary Table: Observed Frequencies			
	IgG beta2-glycoprotein 1 Antibody - negative	**IgG beta2-glycoprotein 1 Antibody - positive >20**	**Row - Totals**
SLE	36	2	38
%	94.74%	5.26%	
SLE + APS syndrome	9	5	14
%	64.29%	35.71%	
Totals	45	7	52
Fisher exact test (1-side)			0.011

In both groups IgM beta2-glycoprotein 1 Antibody is mostly negative. The fisher exact test shows that the IgM beta2-gylcoprotein 1 Antibody is not specific for any patient group (P = 0.275). (Table 10)

Table 10. Observed IgM beta2-glycoprotein 1 antibody frequencies

2-Way Summary Table: Observed Frequencies			
	IgM beta2-glycoprotein 1 Antibody - negative	**IgM beta2-glycoprotein 1 Antibody - positive > 20**	**Row - Totals**
SLE	34	4	38
%	89.47%	10.53%	
SLE + APS syndrome	11	3	14
%	78.57%	21.43%	
Totals	45	7	52
Fisher exact test (1-side)			0,275

In both groups anti-ds DNA antibody is mostly positive. There is no dependence between the anti-ds DNA autoantibody variable and one of the patient groups (P = 0.911). (Table 11)

Table 11. Observed anti-ds DNA antibody frequencies

2-Way Summary Table: Observed Frequencies			
	anti-ds DNA - positive	**anti-ds DNA - negativ (<30)**	**Row - Totals**
SLE	27	17	44
%	61.36%	38.64%	
SLE + APS syndrome	15	10	25
%	60.00%	40.00%	
Totals	42	27	69
Chi-Square test			0.911

It is established that there is no statistically significant difference in complement component levels of C3 and C4 between patients diagnosed with SLE and patients diagnosed with SLE and secondary APS (C3: $P = 0.686$; C4: $P = 0.371$). (Table 12)

Table 12. Difference of C3 and C4 complement levels between Group 1 and Group 2

T-tests; Grouping: Category; Group 1: SLE Group 2: SLE + APS syndrome									
	Mean - SLE	**Std.Dev. - SLE**	**Mean - SLE+ APS syndrome**	**Std.Dev. - SLE+ APS syndrome**	**t-value**	**df**	**p**	**Valid N - SLE**	**Valid N - SLE+ APS syndrome**
C3	0.88	0.26	0.90	0.23	-0.41	72	0.686	45	29
C4	0.14	0.08	0.12	0.07	0.90	72	0.371	45	29

It is established that positive anti-ds DNA antibody titers correlate with a statistically significant difference in C3 and C4 complement component levels in the group of patients with SLE (C3: $P = 0.007$; C4: $P = 0.023$). (Table 13)

Table 13. C3 and C4 complement levels in SLE patients regarding anti-ds DNA titer

Category = SLE T-tests; Grouping: anti-ds DNA; Group 1: positive Group 2: negative (<30)									
	Mean - positive	**Std.Dev. - positive**	**Mean - negative (<30)**	**Std.Dev. - negative (<30)**	**t-value**	**df**	**P**	**Valid N - positive**	**Valid N - negative (<30)**
C3	0.79	0.27	1.01	0.19	-2.84	42	0.007	27	17
C4	0.12	0.08	0.17	0.08	-2.37	42	0.023	27	17

There is no statistically significant difference between anti-ds DNA antibody titers and complement component levels in patients with SLE and secondary APS. (C3: $P = 0.065$; C4: $P = 0.077$). If the p-value was raised to 10% there would be a statistically significant difference. (Table 14)

Table 14. C3 and C4 complement levels in SLE with secondary APS syndrome patients regarding anti-ds DNA titer

Category = SLE + APS syndrome T-tests; Grouping: anti-ds DNA; Group 1: positive Group 2: negative (<30)									
	Mean - positive	**Std.Dev. - positive**	**Mean - negative (<30)**	**Std.Dev. - negative (<30)**	**t-value**	**df**	**P**	**Valid N - positive**	**Valid N - negative (<30)**
C3	0.83	0.20	1.00	0.23	-1.94	23	0.065	15	10
C4	0.11	0.05	0.16	0.09	-1.85	23	0.077	15	10

Amongst patients diagnosed with SLE, there is a statistically significant difference in complement component levels of C3 and C4 in patients with positive IgM aCL antibody titers and negative titers (C3: $P = 0.036$; C4: $P = 0.004$). (Table 15)

Table 15. C3 and C4 complement levels in SLE patients regarding IgM aCL antibody titer

Category = SLE T-tests; Grouping: IgM aCL Antibody; Group 1: negative < 12.5 Group 2: positive									
	Mean - negative < 12.5	**Std.Dev. - negative < 12.5**	**Mean - positive**	**Std.Dev. - positive**	**t-value**	**df**	**P**	**Valid N - negative < 12.5**	**Valid N – positive**
C3	0.94	0.23	0.75	0.31	2.18	38	0.036	28	12
C4	0.17	0.08	0.09	0.07	3.08	38	0.004	28	12

Amongst patients diagnosed with SLE and secondary APS there is no difference in complement component levels in patients with increased IgM aCL antibody titers compared negative titers (C3: $P = 0.656$; C4: $P = 0.526$). (Table 16)

Table 16. C3 and C4 complement levels in SLE with secondary APS syndrome patients regarding IgM aCL antibody titers

Category = SLE + APS syndrome T-tests; Grouping: IgM aCL Antibody; Group 1: negative < 12.5 Group 2: positive									
	Mean - negative < 12.5	**Std.Dev. - negative < 12.5**	**Mean - positive**	**Std.Dev. - positive**	**t-value**	**df**	**P**	**Valid N - negative < 12.5**	**Valid N - positive**
C3	0.91	0.21	0.87	0.21	0.45	23	0.656	18	7
C4	0.12	0.05	0.11	0.05	0.64	23	0.526	18	7

Testing the difference in C3 and C4 complement component levels amongst patients with increased IgG aCL antibody titers and titers within the normal range we can conclude that there is a statistically significant difference for C4 complement levels ($P = 0.004$), while there is no statistically significant difference for C3 complement component levels ($P = 0.052$) in patients diagnosed with SLE. It should be noted that the latter *P*-value of 0,052 almost reaches that of a statistically significant finding of 0.05. (Table 17)

Table 17. C3 and C4 complement levels in SLE patients regarding IgG aCL antibody titer

Category = SLE T-tests; Grouping: IgG aCL Antibody; Group 1: negative Group 2: positive									
	Mean - negative <15	**Std.Dev. - negative <15**	**Mean - positive**	**Std.Dev. - positive**	**t-value**	**df**	**P**	**Valid N - negative <15**	**Valid N - positive**
C3	0.93	0.23	0.75	0.32	2.00	39	0.052	30	11
C4	0.16	0.08	0.10	0.08	2.14	39	0.038	30	11

Testing the difference in C3 and C4 complement component levels amongst patients with increased IgG aCL antibody titers and titers within the normal range we can conclude that there is no statistically significant difference regarding patients with SLE and secondary APS (C3*: P* = 0.471; C4*: P* = 0.058). (Table 18)

Table 18. C3 and C4 complement levels in SLE with secondary APS syndrome patients regarding IgG aCL antibody titers

Category = SLE + APS syndrome T-tests; Grouping: IgG aCL Antibody; Group 1: negative Group 2: positive									
	Mean - negative <15	**Std.Dev. - negative <15**	**Mean - positive**	**Std.Dev. - positive**	**t-value**	**df**	**P**	**Valid N - negative <15**	**Valid N - positive**
C3	0.91	0.22	0.85	0.19	0.73	22	0.471	14	10
C4	0.13	0.04	0.10	0.05	2.00	22	0.058	14	10

We examined the correlation of C3 and C4 complement component levels and anti-ds DNA antibody titers in patients diagnosed with SLE. (Table 19)

This correlation matrix shows a negative and statistically significant correlation of C3 complement component levels and anti-ds DNA antibody titers (r=-0.673; *P* <0.001), and the same applies for the correlation of C4 and anti-ds DNA antibody titers (r=-0.4571; *P* <0.002). This means that high anti-ds DNA antibody titers correlate with lower levels of complement components. (Table 19)

Table 19. Correlation of C3 and C4 complement component levels and anti-ds DNA antibody titer in patients with SLE

Category = SLE Correlations; Marked correlations are significant at p < .05000 N=43	
	anti-ds DNA
C3	-0.6734
	P <0.000
C4	-0.4571
	P =0.002

The correlation is shown graphically in a scatter plot. (Figure 4 and 5)

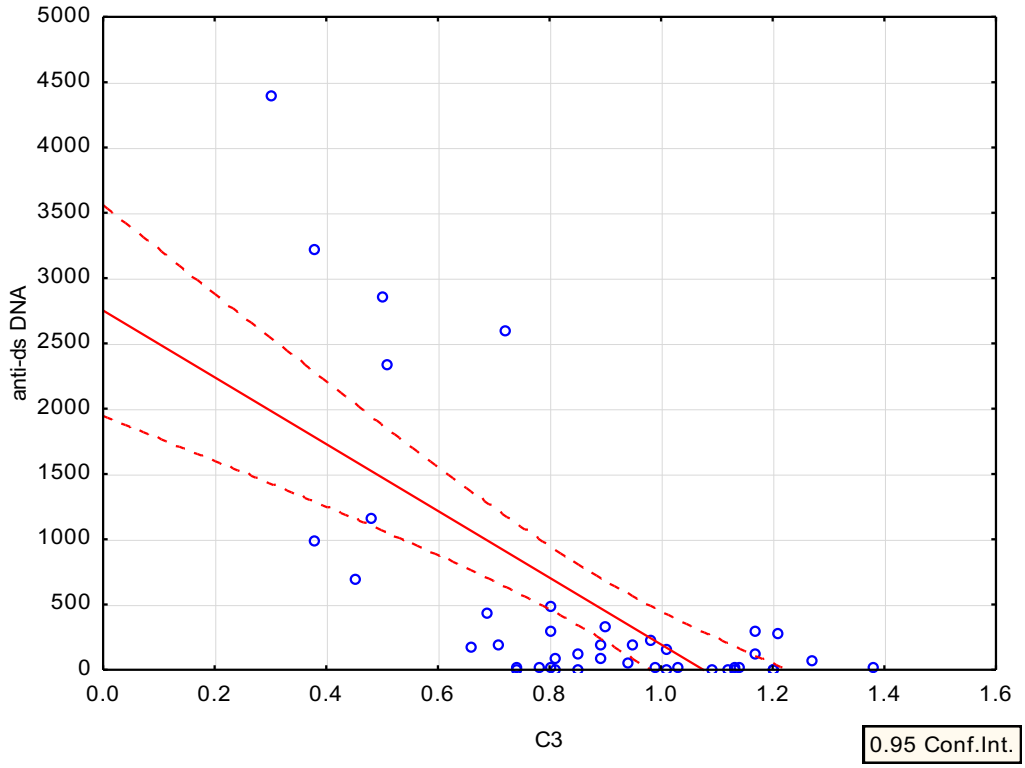

Figure 4. Correlation of C3 and anti-ds DNA in SLE patients

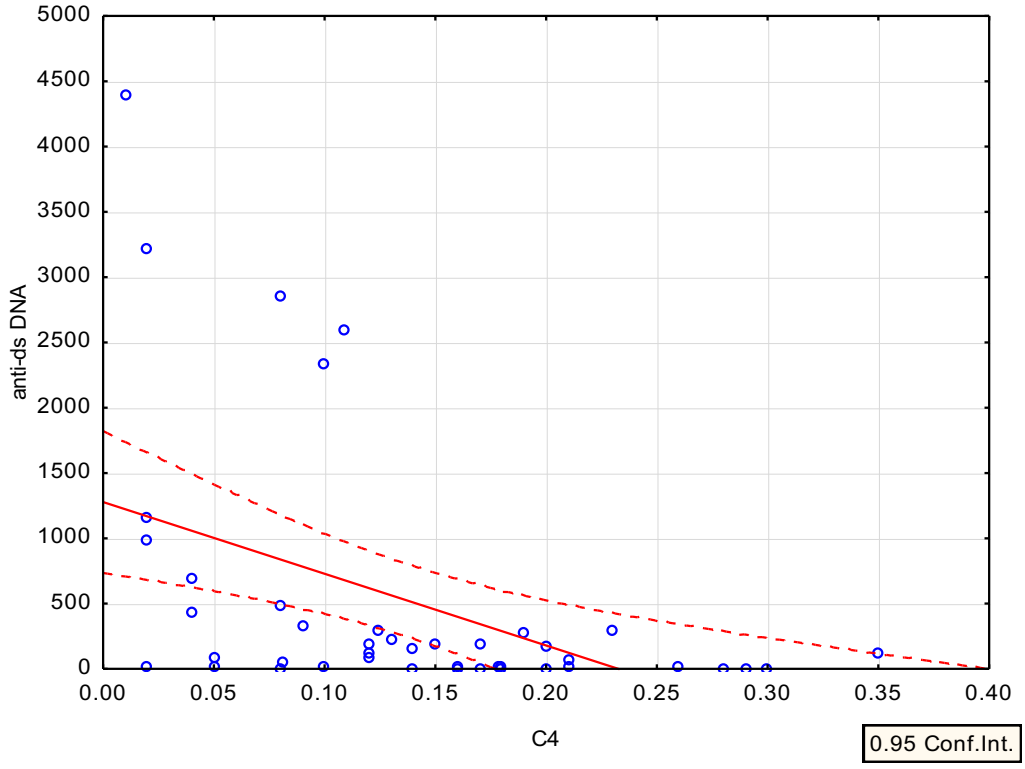

Figure 5. Correlation of C4 and anti-ds DNA in SLE patients

The correlation matrix shows a negative and statistically significant correlation of C4 complement component levels and anti-ds DNA antibody titers (r=-0.4856; *P* = 0.014), while correlation of C3 and anti-ds DNA antibody titers is not statistically significant (*P* <0.052) in patients with SLE and secondary APS. However, it should be noted that the latter *P*-value of 0,052 almost reaches that of a statistically significant finding. (Table 20)

Table 20. Correlation of C3 and C4 complement component levels and anti-ds DNA antibody titer in patients with SLE and secondary APS

Category = SLE + APS Syndrome; Correlations; Marked correlations are significant at p < .05000 N=25	
	anti-ds DNA
C3	-0.3925
	P =.052
C4	-0.4856
	P =.014

The correlation is shown graphically in a scatter plot. (Figure 6)

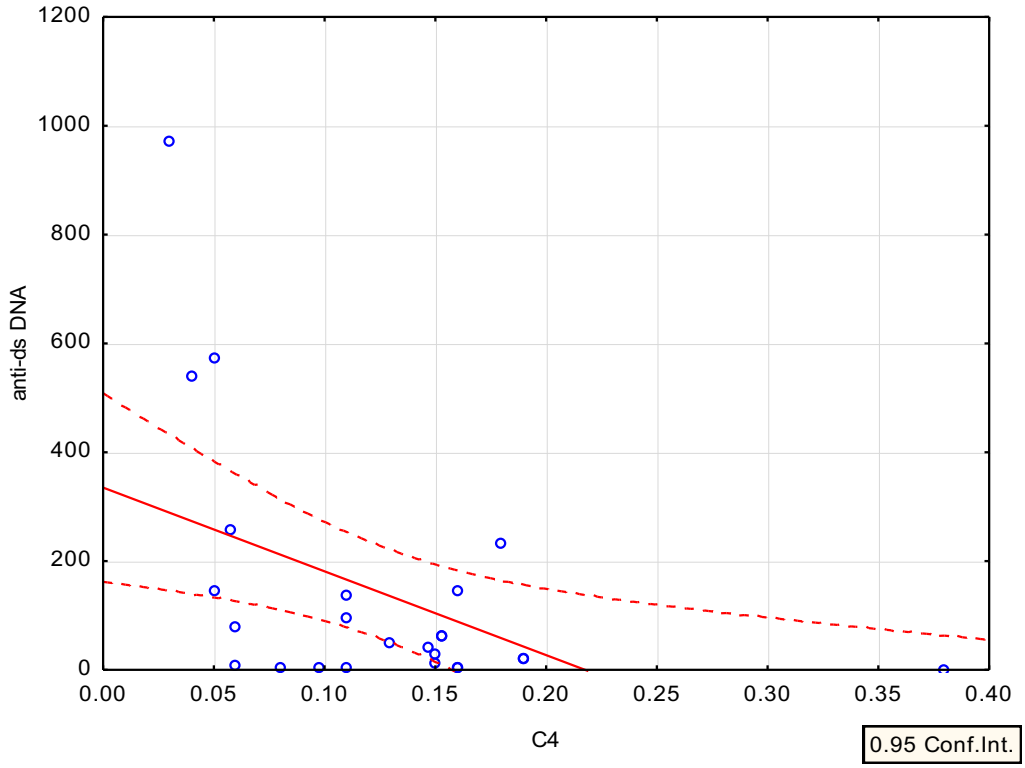

Figure 6. Correlation of C4 and anti-ds DNA in SLE with secondary APS patients

5. DISCUSSION

According to the results obtained in this study, SLE and hence SLE with secondary APS, is more prevalent in the female population, which is consistent with common findings so far (32). In our study mean complement levels of C3 for the group diagnosed with SLE were significantly lowered, while C4 complement component levels in both groups, and C3 levels in the SLE with secondary APS group were found within the reference interval, even though in all cases at the lower end of the normal range. This tendency correlates with other recent findings that stated hypocomplementemia or a tendency for complement components at the lower end of the normal range to be commonly occurring in both APS and SLE (6,18). Consequently, we hypothesized that patients with both SLE and APS have a higher complement consumption and therefore lower complement component levels compared to patients with SLE only. A potentially statistically significant difference might have led us to the assumption that the complement component levels play an even more significant role in the occurrence of SLE with secondary APS compared to SLE alone and this might have emphasized its importance for future diagnostic and therapeutic purposes. Contrary to our hypothesis, there is no statistically significant difference in the complement component levels between the two groups in our study. According to the data collected the complement component levels are almost equal in both groups, with mean levels for C3 of 0.88 and 0.9 and for C4 of 0.14 and 0.12 in patients with SLE and patients with SLE and secondary APS, respectively.

The data of this study also show that in patients diagnosed with SLE positive anti-ds DNA antibody titers correlate with a statistically significant difference in C3 and C4 levels, and this correlation was found to be negative, which means the higher the anti-ds DNA titer, the lower the complement component levels. This correlation is also true for C4 complement component levels and anti-ds DNA titers, but not for C3 levels, however the *P*-value of <0.052 approaches that of a statistically significant finding, in the group of patients with SLE and secondary APS. Other studies have found that high ds-DNA antibody titers and decreased complement levels are more commonly present in SLE flares and active disease compared to remission or inactive disease, which is also points to the importance of appropriate timing when it comes to sampling blood from patients in order to achieve meaningful research results (33). If the same applies for APS and SLE with secondary APS has not been determined yet and could be a topic of future research

The implementation of complement component levels as biomarkers for APS with or without SLE have not been established yet and are the subject of ongoing trials like the PROMISSE study (Predictors of Pregnancy Outcome: Biomarkers in Antiphospholipid Syndrome and Systemic Lupus Erythematosus), which will end in 2019 and will hopefully lead to promising results. Particularly for SLE, only C3 and C4 serum levels have been traditionally used to evaluate the diseases, but recent research has shown that rather the complement products than the substrates, termed serum cell-bound complement activation products, might be more sensitive and specific than conventional serum C3 and C4 levels to assess disease activity. This might also be relevant in prospective research and reveal new insights for the relationship between complement component levels and autoimmune diseases like SLE and APS (34,35).

As discussed beforehand around 30-40% of patients with SLE are aPL positive. One of our aims was to determine the association between aPLs and complement components in SLE without APS and SLE with secondary APS. We found that patients who were diagnosed with SLE without secondary APS and simultaneously had positive aCL titers, had statistically significant lower levels of complement components compared to patients who were aCL negative. These results are consistent with the results of a study conducted 2016 in Norway, that also confirmed significantly lower complement levels in aPL positive patients compared to aPL negative patients (36). This finding may reflect a higher C3 and C4 consumption owing to more prominent complement activation in aCL positive SLE patients compared to aCL negative SLE patients. These results are conceptually in line with our first hypothesis that patients with SLE and secondary APS might have higher complement consumption compared to patients with SLE without secondary APS, even though we could not confirm that hypothesis with our results. The association between complement components and aPLs in patients with SLE and secondary APS has not been a subject of research yet and should be investigated in the future. Our part of the study that dealt with the association between complement components and aPLs in patients with SLE and secondary APS could not produce any statistically significant results. This might also be the result of our study limitations that we will evaluate at the end of this discussion.

Recent research, as outlined in the introduction, has determined that the complement system, especially the anaphylatoxins like C5a and MAC, are key mediators in the pathogenesis of SLE and APS, and some therapeutic approaches targeting complement components, most notably the C5 inhibitor eculizumab, have already been experimentally

proved to work on murine models and have even been successfully conducted in some human cases diagnosed with SLE and APS (18,37–41). These findings emphasize and support the importance of further determining the significance of the complement system for SLE and APS.

There was no information about the current disease activity at the time when the laboratory data included in this cross-sectional study were collected. We could approach this problem by doing a prospective study and collecting blood samples at times of active disease and therefore obtain more consistent, meaningful and comparable data about complement levels. Since both SLE and SLE with secondary APS are rare clinical pictures and Croatia does not offer as many patients as other more populated countries, the small simple size might be a limitation to the validity of our results. In keeping with this limitation, a third control group with for example the diagnosis of primary APS, could yield even more conclusions about the association between complement component levels and specific autoantibodies in SLE and APS. Due to a lack of patients, this could not be accomplished in our hospital setting. Also the incomplete availability of all the variables intended to obtain from eligible patients is a flaw that could be improved in a future study. Ultimately the lack of prior research on the exact topic of this study leaves us without the possibility to compare our results to other study results and the conclusions drawn from this study are therefore limited.

Further research could outline the importance of the complement system and its association with specific autoantibodies in the pathogenesis and occurrence of APS and SLE even more and newly obtained information could eventually be used as a path to new diagnostic criteria, means to monitor the disease and innovative treatment options.

6. CONCLUSION

1. Patients with SLE without APS have statistically significantly lowered C3 complement component levels. Patients with SLE and patients with SLE and secondary APS show a general tendency for complement component levels to be at the lower end of the normal range.

2. In both groups anti-ds DNA antibody was mostly positive. There was no statistically significant difference between anti-ds DNA antibody titers and complement component levels between patients with SLE without secondary APS and patients with SLE and secondary APS.

3. SLE patients have a statistically significant difference in complement levels with positive anti-ds DNA titers. Positive anti-ds DNA titers correlate negatively with C3 and C4 levels in patients with SLE without APS, and the same applied to the C4 levels in patients with SLE and secondary APS.

4. In SLE patients, there was a significant difference in C3 and C4 levels with positive IgM aCL antibody titers, while for IgG aCL antibody titers, the same applied to C4 complement levels.

Some of the results are consistent with published reports so far, while other relations are surprising. Particularly interesting are the correlations between complement and anticardiolipin antibodies, most notably IgM, that suggest a complex role of the complement system in autoimmune events. We hold that we have highlighted the complexity of complement component levels in regard to diseases such as SLE and SLE with secondary antiphospholipid syndrome, especially in relation to specific autoantibodies.
Further research with an improved study design that overcomes the limitations described beforehand is necessary in order to yield more insight about the role of complement or complement activation products and their relation to specific antibodies and the course of SLE and APS.

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8. SUMMARY

Objectives: To compare levels of complement components between patients with SLE and patients with SLE and secondary antiphospholipid syndrome and investigate their relation with levels of anti-dsDNA and anticardiolipin antibodies.

Materials and methods: A cross-sectional study was conducted at the Department of Rheumatology and clinical Immunology of the University Hospital Split (KBC Split). Included were 74, 45 with SLE and 29 with SLE and secondary APS. Patient data were obtained from the database at the Department for Rheumatology and Immunology as well as at the Institute for Laboratory Diagnostics, KBC Split. The investigated laboratory parameters are: antinuclear antibodies, anti-ds DNA antibodies, C3, C4, IgG, IgM, IgA, Lupus Anticoagulant, IgG and IgM anticardiolipin antibodies, IgG and IgM beta2-gylcoprotein 1 antibodies.

Results: All patients with SLE had decreased C3 values, while the C4 values were within the reference interval. Patients with SLE and secondary APS had C3 and C4 levels within the reference interval but at the lower limit. In both groups, aCL IgG and IgM antibodies were mostly negative, whereas anti-ds DNA antibodies were mostly positive. Anti-ds DNA was statistically significantly correlated with C3 (P <0.001) and C4 (P <0.002) in SLE patients without APS, the same correlation was confirmed in patients with SLE and secondary APS for C4 complement levels (P = 0.014). Only the group of patients with SLE without secondary APS show a correlation of C3 and C4 with a positive titer of IgM aCL antibodies (C3: P = 0.036; C4: P = 0.004) as well as C4 with IgG aCL antibodies (P = 0.004). There is no statistically significant difference in complement component levels of C3 and C4 between patients diagnosed with SLE without APS and patients diagnosed with SLE and secondary APS (C3: P = 0.686; C4: P = 0.371).

Conclusion: In SLE patients, lowered levels of C3 were confirmed as well as the correlation of reduced complement levels with the specific autoantibody dsDNA**.** The expected difference between C3 and C4 complement levels was not confirmed between patients with SLE without APS and patients with SLE and secondary APS. Interesting are the correlations of complement and anticardiolipin antibodies, in particular IgM, that suggest a complex role of complement in autoimmune events and the need for further research.

9. CROATIAN SUMMARY

Naslov: USPOREDBA RAZINA KOMPONENTI KOMPLEMENTA U SISTEMSKOM LUPUSU I SEKUNDARNOM ANTIFOSFOLIPIDNOM SINDROMU U ODNOSU NA SPECIFIČNA AUTOPROTUTIJELA

Ciljevi: Usporediti razine komponenti komplementa između bolesnika sa sistemskim lupusom (SLE) i bolesnika sa SLE i sekundarnim antifosfolipidnim sindromom te istražiti njihovu povezanost s razinama anti-dsDNA i antikardiolipinskih protutijela.

Materijali i metode: Presječna studija provedena je u Zavodu za reumatologiju i kliničku imunologiju KBC-a Split. Uključena su 74 bolesnika, 45 sa SLE i 29 sa SLE i sekundarnim APS. Podatci o bolesnicima dobiveni su iz baze podataka Zavoda za reumatologiju i imunologiju te Zavoda za laboratorijsku dijagnostiku, KBC Split. Istraživani laboratorijski parametri su: antinuklearna antitijela, anti-ds DNA antitijela, C3, C4, IgG, IgM, IgA, Lupus antikoagulans, antikardiolipinska protutijela klase IgG i IgM,beta2-glikoprotein protutijela klase IgG i IgM.

Rezultati: Svi bolesnici sa SLE imali su snižene vrijednosti C3 dok su vrijednosti C4 bile unutar referentnih vrijednosti. Bolesnici sa SLE i sekundarnim APS imali su C3 i C4 unutar referentnih vrijednosti ali na donjoj granici. U obje skupine aCL IgG i IgM protutijela su bila većinom negativa dok su anti-ds DNA protutijela bila većinom pozitivna. Anti-ds DNA su statistički značajno korelirala s razinama C3 (P <0.001) i C4 (P <0.002) u skupini SLE bolesnika bez APS, ista korelacija je potvrđena u bolesnika sa SLE i sekundarnim APS za razinu C4 (P = 0.014). Također je samo u skupini bolesnika sa SLE bez APS potvrđena značajna korelacija C3 i C4 s pozitivnim titrom IgM a CL protutijela (C3: P = 0.036; C4: P = 0.004) kao i IgG aCL protutijela samo s titrom C4 (P = 0.004). Nije potvrđena statistički značajna razlika u razinama C3 i C4 bolesnika sa SLE bez APS i SLE sa APS (C3: P = 0.686, C4: P = 0.371).

Zaključci: U bolesnika sa SLE potvrđene su snižene razine C3 te povezanost sniženih razina komplementa sa specifičnim autoprotutijelom ds DNA. Nije potvrđena očekivana razlika u razinama C3 i C4 između bolesnika sa SLE bez APS i bolesnika sa SLE i APS. Zanimljive su korelacije komplementa s antikardiolipinskim protutijelima, posebice IgM što upućuje na složenu ulogu komplementa u autoimunim zbivanjima i potrebu daljnjih istraživanja.

10. CURRICULUM VITAE

Personal information:

Name: Bernarda Crnjak

Date & place of birth: 6th January 1992, in Bielefeld, Germany

Nationality: Croatian

Address: Poljička 12 in 21000 Split, Croatia

E-mail: bernarda.crnjak@gmail.com

Education:

Since October 2012: Medical Studies at the University of Split School of Medicine

July 2011: Abitur at Ratsgymnasium in Bielefeld, Germany