Systemic lupus erythematosus : from diagnosis to prognosis

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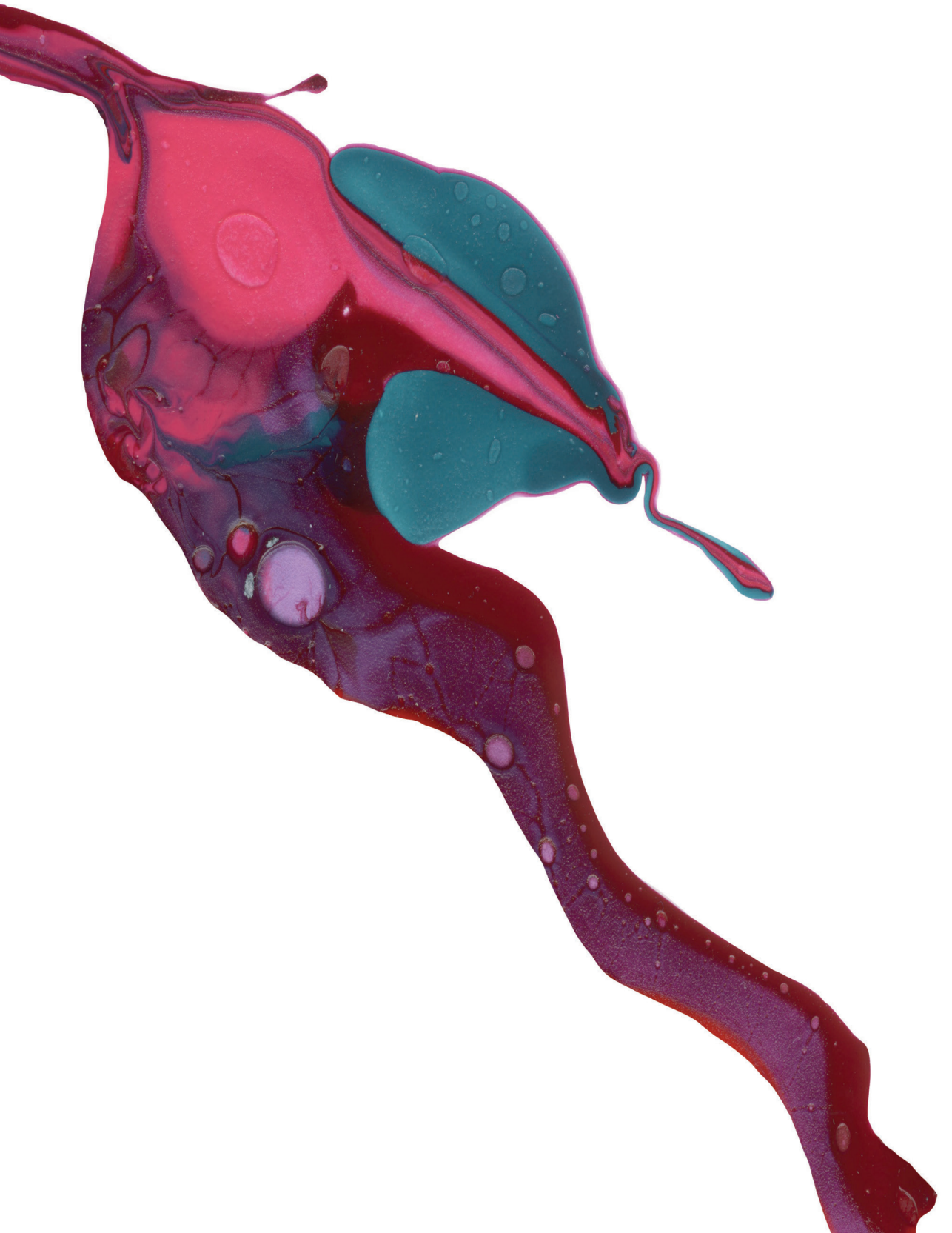


Chapter 1

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

PROLOGUE: HIPPOCRATES' LEGACY

"The physician must be able to tell the antecedents, know the present, and foretell the future – must mediate these things, and have two special objects in view with regard to disease, namely, to do good or to do no harm."

So wrote Hippocrates – the father of modern Western medicine – in his monumental work *Epidemics*. Ever since there have been doctors caring for ill patients, the most pertinent question has been: will the patient get better, and – if yes – by what therapeutic means? Prognosis and treatment: they are fundamental pillars in medicine, reflecting immediate needs of the ill patient. However, the first step towards successfully treating an illness is finding out what is wrong. This process referred to as "diagnosis" is derived from the Greek word διαγιγνώσκειν, meaning "to discern, or to distinguish".

Diagnosis

Making a diagnosis involves the identification of the nature and cause of a certain phenomenon. Accordingly, patients are categorised as having a disease based on a common **aetiology**, **pathogenesis**, or **symptoms**.

In the early days of medicine, the limited availability of diagnostic tools challenged the process of making a diagnosis. Hippocrates used whatever he could take in from his environment in making a diagnosis: from dietary habits, the season, prevailing winds, the water supply at the patient's home, to the tasting of urine and smelling of sweat.[1] In modern medicine, more advanced diagnostic methods have come at hand. In spite of these advanced methods, the process of diagnosis may currently still be challenging. First, doctors must agree on a definition of a specific disease. However, diagnosis and disease do not represent an unchangeable truth: with expanding knowledge about disease mechanisms and increasingly sophisticated diagnostic tools, definitions and boundaries of disease continue to shift. These shifting disease definitions do not only reflect advancing knowledge, but also the need for doctors to incorporate developments with a practical means to group patients assisting in clinical decision making. In some instances, the discovery of a mutation in a single gene associated with a consistent phenotype results in a clear-cut definition of disease and a corresponding gold standard diagnostic test, as is the case in, for instance, cystic fibrosis and sickle cell disease. However, in most instances the cause is not found in a single gene, but seems to be a complex multifactorial interplay between genes and environment. In more complex cases, a clear-cut definition and gold standard diagnostic tool may never be found.

In making a diagnosis, the underlying concept of disease as a dichotomous state is challenged by the nature of many diseases. The clinical symptoms and laboratory indicators chosen to define a disease in reality represent a continuum. Disease is usually acquired by

degrees, starting with exposure to a particular risk factor, followed by the development of subclinical pathologic changes and evolution of symptoms and signs. Clearly, there is a smooth transition from low to high values of the diagnostic indicators with increasing degrees of dysfunction. Thus, most distributions of clinical variables are not easily divided into normal and abnormal. In reality, most diagnoses are defined by a cut-off, chosen at some point in the continuum between health and disease. This cut-off may be based on a laboratory abnormality, but may also more abstractly be seen as a combination of different laboratory test results and clinical signs and symptoms, of which certain sets have to be present to meet the diagnosis. If the cut-off of the diagnostic indicator is set too high, patients with the disease may be missed, thereby decreasing the sensitivity of the indicator. However, if the cut-off is set too low, individuals without the disease may be incorrectly identified as being ill, decreasing the specificity of the diagnostic indicator. In formulating disease definitions, there is a constant trade-off between sensitivity and specificity.

Prognosis

Traditionally, diagnosis is seen as the primary guide to treatment and prognosis. However, the continuum between health and disease does not resolve once a certain cut-off is chosen and a patient fulfils the criteria for a particular diagnosis. Ultimately, prognostic implications are of primary importance to the patient. Because patients diagnosed with a disease may have various degrees of the disease, there is a need to stratify patients by prognosis. Prognosis, derived from the Greek "πρόγνωσις" (fore-knowing, foreseeing), is the likelihood of future outcomes in a patient with a given disease. Predicting an individual's prognosis can involve a wide range of relevant and available information, including disease, patient, demographic, and socioeconomic factors. Thus, prognosis offers an alternative starting point with wider incorporation of factors relevant to patient outcomes than diagnosis alone. By this approach, multiple "sub-diagnoses" of prognostic subgroups can be made among patients with a given diagnosis. Personalised medicine, also coined precision medicine, patient-tailored medicine, or stratified medicine, is the approach in clinical medicine that attempts to incorporate the prognosis of individual patients. The goals of personalised medicine are to optimise treatment efficacy for the individual patient, and to minimise the risk of adverse effects due to ineffective treatment.

In this thesis, issues relating to the diagnosis and prognosis of systemic lupus erythematosus (SLE) were investigated, a condition in which the aforementioned understanding of disease as a clinical and pathologic spectrum with prognostic subgroups is clear. In the first part of this introduction, SLE as a heterogeneous disease will be portrayed, demonstrating areas from which the challenges in diagnosis and prognosis arise. In the second part, two of the most severe visceral manifestations of SLE – lupus nephritis and neuropsychiatric lupus – will illustrate that the concept of SLE as a spectrum extends to these specific organ manifestations. Specific challenges in the accurate diagnosis and prognosis of these manifestations will be pointed out.

PART 1: SYSTEMIC LUPUS ERYTHEMATOSUS

SLE is an autoimmune disease characterised by loss of tolerance against nuclear autoantigens, lymphoproliferation, production of autoantibodies, immune complex disease, and multiorgan tissue inflammation.[2] SLE is a systemic disorder that ranges from a limited cutaneous disorder to life-threatening multisystemic disease with major organ involvement. The heterogeneous manifestations of SLE and the overlap of symptoms with various other diseases can make its diagnosis extremely challenging. Because SLE may be accompanied by significant morbidity that can be fatal, prompt diagnosis and subsequent selection of the most effective therapy is of utmost importance for patients suffering from this disease. Here, diagnostic and prognostic challenges in SLE will be demonstrated by review of the clinical heterogeneity and aetiopathogenic complexity of this disease. First, review of the history will reveal how SLE with its myriad manifestations became established as an entity over time.

Historic background

Disease definitions of SLE have shifted considerably throughout history, reflecting advancing knowledge and increasingly sophisticated diagnostic tools. The term "lupus" (Latin for "wolf") was first used during the Middle Ages to describe erosive skin lesions reminiscent of a wolf's bite.[3] The first clear description of lupus is credited to Biett of the Paris School of Dermatology, referring to centrifugal erythema.[4] His student, Cazenave, published Biett's work and coined the term "lupus érythémateux" (lupus erythematosus) in 1833. Cazenave classically described lupus as a rare condition, appearing most frequently in young females who were otherwise healthy, mainly affecting the face. In 1846, the Viennese physician Ferdinand von Hebra introduced the butterfly metaphor to describe the malar rash characteristic of SLE.[5] He and his son-in-law Moritz Kaposi also first recognised lupus as having a cutaneous form as well as a systemic form characterised by subcutaneous nodules, arthritis with synovial hypertrophy of both small and large joints, lymphadenopathy, fever, weight loss, anaemia, and central nervous system involvement.[6] Over the next thirty years, pathologic studies recognised the existence of nonbacterial verrucous endocarditis (Libman-Sacks endocarditis),[7] and wire-loop lesions in individuals with glomerulonephritis.[8]

Osler wrote three papers during the years 1895–1904 in which he described the visceral complications of lupus erythematosus with cutaneous involvement. Retrospectively, only two out of the 29 patients he described definitely appear to have had SLE, while the other patients likely suffered from Henoch-Schönlein purpura and a number of other conditions.[9] Clearly, the early diagnosis of SLE depended largely on the finding of skin lesions, which may show overlap with currently known conditions including cutaneous tuberculosis, syphilis, vasculitis, and others. The introduction of antibiotic treatment assisted in the distinction

of patients with true SLE from those with confounding conditions. In 1948, the ability to more specifically define SLE allowed Hargraves and colleagues to study bone marrow preparations of 25 patients with SLE in which they revealed nuclei derived from dead cells that were phagocytosed by mature polymorphonuclear cells and were being digested. This phenomenon resulted in a distinct appearance by light microscopy coined the "LE cell".[10] In 1954, Miescher and Fauconnet observed that when serum from SLE patients was incubated with a suspension of cell nuclei, the ability to induce the LE cells was eliminated. This observation indicated that either the serum factor responsible for the LE cell phenomenon was destroyed by exposure to the nuclei, or removed from the serum by reacting with the nuclei.[11] In 1958, Friou demonstrated that the serum factor of patients with SLE that reacted with the nuclei of cells was gamma globulin, and the target in the nucleus was DNA forming complexes with histones.[12] The serum factor was called the "antinuclear factor" and could be detected by an indirect immunofluorescence technique. These observations in the late 1950s clearly demonstrated an autoimmune pathogenic process underlying SLE, and paved the way for the modern era of diagnosing SLE.

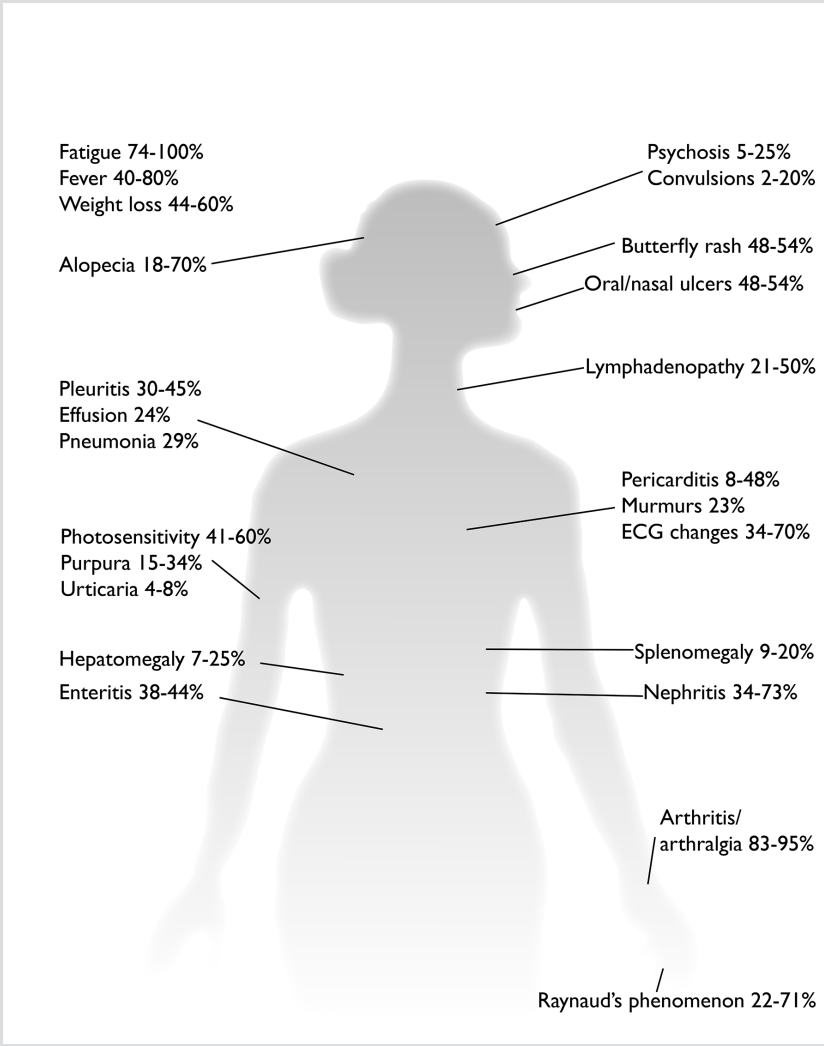
Epidemiology and symptomatology

In modern times, the reported prevalence of SLE ranges from 20 to 150 cases per 100,000.[13] SLE predominantly affects women, with a male-to-female ratio ranging from 1:3 in children, 1:7–15 in adults, and 1:8 in older individuals.[14, 15] The peak age of onset of SLE is between 20–40 years of age.[13] African Americans and Hispanics are affected more frequently than Caucasians, and have higher morbidity.[13]

SLE can affect any part of the body, as demonstrated by its numerous clinical manifestations. The most common clinical signs and symptoms of SLE are shown in **Figure 1**.

Because of its heterogeneous manifestations, SLE often mimics other diseases. Therefore, SLE is frequently coined the "great imitator", and is thereby a classic diagnostic consideration in the differential diagnosis of many diseases. Since diagnostic criteria for SLE are lacking, the diagnosis of SLE is usually made clinically after exclusion of other diagnoses. In clinical practice, classification criteria for SLE are frequently used as diagnostic criteria, although they serve a different purpose (**Box 1**).

Figure 1 Clinical manifestations of SLE.



Percentages indicate the frequency of the manifestation occurring at any time in the disease course. Adapted from: Von Feldt J.M.[16]

Box 1.

Diagnostic versus classification criteria

Diagnostic criteria are a set of signs, symptoms, and tests for use in routine clinical practice to guide the care of individual patients.[17] To be successful, diagnostic criteria must reflect the broad spectrum of different features of a disease, with the objective to accurately identify as many patients with the disease as possible, including those with atypical phenotypes of the disease. In contrast, classification criteria are primarily intended to create well-defined, homogeneous cohorts of patients for clinical research. Classification criteria do not aim to capture the entire population of possible patients, but rather to capture the majority of patients with the principal features of the condition. Hence, the goal of classification criteria differs from the goal of diagnostic criteria. As a consequence, classification criteria generally tend to be more specific, but less sensitive than diagnostic criteria. Because SLE is a very heterogeneous disease and a gold standard for its diagnosis is lacking, diagnostic criteria with sufficient sensitivity and acceptable specificity remain unattainable. Although SLE classification criteria may support a diagnosis of SLE, clinicians today are still compelled to diagnose SLE based upon the totality of patients' disease manifestations.

Classification of SLE

An accurate and validated set of classification criteria is critical to the interpretation of study findings and to the comparison of results between studies. Existing classification criteria for SLE have traditionally been developed with the aim to distinguish patients with SLE from patients with various other diseases that are mainly encountered in rheumatology clinics.[18-20] In the development of the 1982 and 1997 American College of Rheumatology (ACR) and 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE, the approach has been to include a cohort of patients recruited from rheumatology clinics with putative SLE as well as with other diseases that might appear in the differential diagnosis in that setting. In the process of the derivation of the set of classification criteria, experienced rheumatologists – assisted by mainly dermatologists and neurologists – then reached a consensus on a "reference-standard" clinical diagnosis for each patient in the cohort. Subsequently, various combinations of classification criteria were tested to investigate which have the optimal sensitivity and specificity to distinguish patients with SLE from those with other rheumatic diseases while at the same time do not falsely classify patients with other rheumatic diseases as having SLE.

The original criteria for the classification of SLE established by the American Rheumatism Association (ARA) in 1971 have been revised in 1982 by Tan *et al.*[20] and updated by Hochberg *et al.*[18] in 1997 under the auspices of the ACR, resulting in a list of 11 items (**Table 1**). Accordingly, a patient can be classified as having SLE if any four or more of the criteria are present, serially or simultaneously, during any interval of observation.

These ACR criteria were long used as inclusion criteria for clinical trials involving patients with SLE. When tested against patients with other rheumatic diseases in the derivation cohort of this classification, these criteria had a sensitivity of 86% and a specificity of 93%.[20] However, over the years a general consensus was reached that the ACR criteria over-represent cutaneous lupus, may not capture early lupus, and do not capture some patients with lupus nephritis and neuropsychiatric lupus.[21]

Table 1 ACR classification criteria for SLE.[18, 20]

Criterion	Definition
1. Malar Rash	Fixed erythema, flat or raised
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight
4. Oral ulcers	Oral or nasopharyngeal ulceration
5. Nonerosive arthritis	Involving two or more peripheral joints, characterised by tenderness, swelling, or effusion
6. Serositis	Pleuritis: typical pleurisy or pleural rub or evidence of pleural effusion *or* Pericarditis: documented by electrocardiogram or pericardial rub or evidence of pericardial effusion
7. Renal disorder	Persistent proteinuria >0.5 grams per day or >3+ *or* Cellular casts (red cell, haemoglobin, granular, tubular, or mixed)
8. Neurologic disorder	Seizures (*in the absence of other causes*) *or* Psychosis (*in the absence of other causes*)
9. Hematologic disorder	Haemolytic anaemia *or* Leukocytopenia (<4.0*10^9/L on ≥2 occasions) *or* Lymphocytopenia (<1.5*10^9/L on ≥2 occasions) *or* Thrombocytopenia (<100*10^9/L in the absence of offending drugs)
10. Immunologic disorder	Anti-dsDNA: antibody to native DNA in abnormal titre *or* Anti-Sm: presence of antibody to Sm nuclear antigen *or* Positive finding of antiphospholipid antibodies: • an abnormal serum level of IgG or IgM anticardiolipin antibodies *or* • a positive test result for lupus anticoagulant using a standard method *or* • a false-positive test result for at least six months confirmed by Treponema pallidum immobilisation or fluorescent treponemal antibody absorption test
11. Antinuclear antibody	An abnormal titre of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs

A group of experts on SLE, unified as the SLICC, more recently proposed revised criteria for SLE (**Table 2**).[19] In order for a patient to be classified as SLE according to the SLICC criteria requires either that the patient fulfils at least four of 17 criteria, including at least one of the 11 clinical criteria and one of the six immunologic criteria, or that the patient has biopsy-proven nephritis compatible with SLE in the presence of antinuclear antibodies (ANA) or anti-double-stranded DNA (dsDNA) antibodies.

The SLICC criteria were validated in 690 patients with SLE and other rheumatic diseases. The SLICC revised criteria had a greater sensitivity but lower specificity than the 1997 ACR classification criteria (sensitivity of 97 vs. 83 percent and specificity of 84 vs. 96 percent, respectively).[19]

Reflected by the high but still suboptimal sensitivity of the different SLE classification criteria, some patients are clinically diagnosed with SLE without fulfilling SLE classification criteria. Sometimes these patients are designated as having "incomplete" or "latent" SLE, and may

presumably be encountered at a relatively early time point in the course of their disease. A substantial proportion of these patients may develop "complete" SLE according to classification criteria during follow-up,[22] particularly in the presence of discoid lupus, positive anti-dsDNA and/or anti-Sm antibodies.[23]

Aetiology

Making a diagnosis based on clinical symptoms alone may adversely affect outcomes by obscuring important information about the aetiology and pathogenesis of the disease. For instance, infectious diseases such as borreliosis, leishmaniasis, and those caused by parvovirus and human immunodeficiency virus may mimic systemic symptoms of SLE, and may even result in a patient fulfilling clinical classification criteria for SLE.[24-27] However, the underlying aetiology and pathogenesis of these conditions are different and thereby the indicated treatment and outcome are also expected to be different. As seen in the classification criteria for SLE, the demonstration of antinuclear autoantibodies plays a central role in the diagnosis of SLE. Antinuclear autoantibodies are omnipresent in SLE and are typically present many years before the clinical diagnosis with a progressive accumulation of specific autoantibodies.[28] However, the aetiology of the break in tolerance and subsequent production of autoantibodies in SLE is not clear-cut and appears to be multifactorial. Multiple genetic predispositions and gene-environment interactions have been identified in the setting of SLE. A longstanding proposed mechanism for the development of autoantibodies involves a dysregulation of various cell death processes (including apoptosis, necrosis, and NETosis[29]) with defective clearance of dying cells. Either there is excess cell death or failure to clear debris from dying cells efficiently. Exposure of the immune system to these hidden antigens can result in a break of tolerance and an autoimmune response directed to these nuclear antigens – ultimately resulting in the production of autoantibodies characteristic of SLE. Excess exposure to these nuclear antigens can be seen in the case of exposure to ultraviolet radiation,[30] and mass cell death associated with physiologic processes, or effects of viruses and medication. Defective clearance of debris from dying cells consisting of antigens that are normally hidden from the immune system, such as nuclear antigens (chromatin and histone proteins) and components of cell membranes (phospholipids), can occur with e.g. defective phagocytosis, a deficiency of early complement components,[31] and defective DNase.[32] In addition, aberrant antigen presentation and defects in T and/or B cell selection or regulation may be involved in the development and perpetuation of autoimmunity in SLE.[33]

The high concordance rate of SLE among monozygotic twins[34] and the increased risk of SLE in first-degree relatives[35] suggest a strong genetic component in the aetiology of SLE. Genome-wide association studies (GWAS) have identified approximately 50 genes that predispose to SLE.[36] However, these genes account for only a limited part of susceptibility to SLE, suggesting a large influence of environmental factors. The most common genetic predisposition for SLE is found at the locus of the major histocompatibility complex (MHC).

Table 2 SLICC classification criteria for SLE.[19]

Clinical criteria	
1. Acute cutaneous lupus	a. Lupus malar rash (*not if discoid*) *or* b. Bullous lupus *or* c. Toxic epidermal necrolysis variant of SLE *or* d. Maculopapular lupus rash *or* e. Photosensitive lupus rash (*in the absence of dermatomyositis*) *or* f. Subacute cutaneous lupus
2. Chronic cutaneous lupus	a. Classic discoid rash localised/generalised *or* b. Hypertrophic (verrucous) lupus *or* c. Lupus panniculitis (profundus) *or* d. Mucosal lupus *or* e. Lupus erythematosus tumidus *or* f. Chilblain lupus *or* g. Discoid lupus/lichen planus overlap
3. Oral/nasal ulcers	Oral or nasopharyngeal ulceration *(In the absence of vasculitis, Behçet's disease, infection (herpes), inflammatory bowel disease, or acidic foods)*
4. Non-scarring alopecia	Diffuse thinning or hair fragility with visible broken hairs *(In the absence of other causes such as alopecia areata, drugs, iron deficiency, and androgenic alopecia)*
5. Synovitis (≥2 joints)	a. Swelling or effusion *or* b. Tenderness in ≥2 joints and at least 30 minutes of morning stiffness
6. Serositis	a. Typical pleurisy >1 day *or* pleural effusion *or* pleural rub *or* b. Typical pericardial pain >1 day *or* pericardial effusion *or* pericardial rub *or* pericarditis by electrocardiography *(In the absence of other causes, such as infection, uraemia, or Dressler's pericarditis)*
7. Renal disorder	a. Urine protein-to-creatinine ratio (or 24-hour urine protein) representing 500 mg protein/24 hours *or* b. Red blood cell casts
8. Neurologic disorder	a. Seizures *or* b. Psychosis *or* c. Mononeuritis multiplex *(in the absence of other known causes such as primary vasculitis*) *or* d. Myelitis *or* e. Peripheral or cranial neuropathy (*in the absence of other known causes such as primary vasculitis, infection, and diabetes mellitus*) *or* f. Acute confusional state (*in the absence of other causes, including toxic/metabolic, uraemia, drugs*)
9. Haemolytic anaemia	
10. Leukocytopenia/ lymphocytopenia	a. Leukocytopenia: at least once (<4000/mm^3) (*In the absence of other known causes such as Felty's syndrome, drugs, and portal hypertension*) *or* b. Lymphocytopenia: at least once (<1000/mm^3) (*In the absence of other known causes such as corticosteroids, drugs, and infection*)
11. Thrombocytopenia	At least once (<100,000/mm^3) (*In the absence of other known causes such as drugs, portal hypertension, and thrombotic thrombocytopenic purpura*)

Table 2 Continued.

Immunologic criteria	
1. Antinuclear antibody	Level above the laboratory reference range
2. Anti-dsDNA	Antibody level above laboratory reference range (or 2-fold the reference range if tested by ELISA)
3. Anti-Sm	Presence of antibody to Sm nuclear antigen
4. Antiphospholipid antibody	a. Positive test for lupus anticoagulant b. False-positive test for rapid plasma reagin c. Medium- or high-titre anticardiolipin antibody level (IgA, IgG, or IgM) d. Positive test result for anti-2-glycoprotein I (IgA, IgG, or IgM)
5. Low complement	C3, C4, or CH50
6. Direct Coombs' test	*In the absence of haemolytic anaemia*

T cells and B cells recognise self and foreign peptides presented on the cell surface by human leukocyte antigens (HLA) encoded by genes of the MHC. A breakdown of immunologic tolerance to self-antigens may be mediated by aberrant presentation of self or foreign peptides to autoreactive T cells via HLA molecules. Notably, the HLA genes HLA-A1, -B8, and -DR3 have been linked to SLE.[37]

It is thought that in certain individuals with a genetic immunologic background that predisposes them to SLE, exposure to a specific environmental factor may trigger SLE. Possible triggers may include exposure to sunlight, infection, surgery, or pregnancy. The observations that (i) SLE is a disease predominantly affecting women; (ii) SLE particularly affects women of fertile age; and (iii) SLE may flare during pregnancy,[38] implicate pregnancy as an intriguing aetiologic factor. Possibly, the antibodies in SLE are not only directed to nuclear autoantigens, but also to nuclear antigens derived from chimeric cells that are acquired during pregnancy. In this thesis, the relationship between pregnancy, chimerism, and SLE was investigated further.

Pregnancy-derived chimerism in SLE

In the 1990s, the discovery of bi-directional cell trafficking during human pregnancy resulting in the persistence of fetal cells in the mother and of maternal cells in her offspring for decades after birth shed new light on the relationship between pregnancy and SLE.[39] Consequently, a role for pregnancy-acquired chimerism as an aetiologic factor in SLE was postulated, which was investigated further in this thesis.

The term chimerism stems from the Greek mythical beast "chimera" (Χίμαιρα): a creature with the head of a lion, the body of a goat, and the tail of a dragon. In medicine, (micro) chimerism refers to the occurrence of (small) numbers of cells of a distinct genetic constitution in an individual.

Pregnancy is presumed to be the most important physiologic source of microchimerism in women. During pregnancy, fetal cells can enter the maternal circulation across the placenta (fetal microchimerism), and maternal cells can enter the fetal circulation *vice versa* (maternal microchimerism). A pregnant woman can acquire chimeric cells from the fetus as a consequence of completing pregnancy, but chimeric cells may also be exchanged during a miscarriage or abortion.[40, 41] Transplacental exchange of cells is possible because the placenta contains microscopic disruptions, which become more permeable as pregnancy progresses.[42] Implantation of embryonic chorionic villi in the functional endometrium results in a primitive fetomaternal circulation by the end of the third week of embryonic development.[43] As pregnancy progresses, the placental barrier becomes increasingly thinner, while fetal blood flow and blood pressure increase, and the villous tree expands.[42] Small quantities of chimeric cells (ranging from 1 to up to 400 per 10^6 cells) can be detected in nearly all pregnant women, starting as early as 4 weeks after gestation.[44] Fetal chimeric cells may be present in mothers as hematopoietic progenitor cells, trophoblast cells, nucleated erythrocytes, T lymphocytes, as well as other leukocytes.[45-52] Pregnancy has also been shown to leave a long-term legacy: chimeric cells may persist in healthy women for up to 27 years after pregnancy.[39]

While microchimerism during pregnancy is common in healthy individuals and may even assist in tissue repair and maintenance, fetal microchimerism has been implicated in various adverse phenomena, including autoimmune disease, pregnancy complications, malignancy, infectious disease and the production of donor-specific antibodies in the setting of organ transplantation. Maternal microchimerism is studied less frequently, but may be of pathogenic significance in neonatal lupus syndrome,[53] juvenile idiopathic inflammatory myopathies,[54] and juvenile dermatomyositis.[55] Few studies have looked at microchimerism in relation to SLE. Typically, studies have focused on the detection of male microchimerism of presumably fetal origin as identified by the Y chromosome in whole blood or in tissues of female patients. At the tissue level, an increased occurrence of male microchimerism has been demonstrated in women with SLE as compared to healthy women.[56-58] A number of studies have shown that in peripheral blood, there is also an increased frequency of male microchimerism in SLE patients compared to controls,[59, 60] whereas other studies have found no difference.[61, 62] Interestingly, although pregnancy is presumed to be the main source of chimerism in these cases, a clear-cut relationship between microchimerism in tissues and pregnancy was not found. To substantiate ongoing research in the field of chimerism and SLE and other autoimmune diseases with a female preponderance, the significance of chimerism in pregnancy must be established first.

A crucial finding pointing towards a role for chimerism in SLE came from a mouse model for graft-versus-host disease developed by Via and Shearer in the late 1980s.[63] In this model, the injection of a specific type of parental T lymphocytes into F1 recipients resulted in a condition resembling human SLE. This was accompanied by proliferative glomerulonephritis with deposition of immune complexes similar to lupus nephritis, lymphoid hyperplasia,

and production of antibodies against nuclear antigens, erythrocytes, and thymocytes. In this model, donor parental T helper cells were able to continuously stimulate F1 host B cells because of a low frequency of cytotoxic T cell precursors. This landmark study led to the hypothesis that a graft-versus-host phenomenon may also be involved in human SLE, as well as in other autoimmune diseases. In this setting, the host must accept the presence of chimeric cells, chimeric cells must be immunocompetent T cells, chimeric cells must recognise the host as foreign, and there must be a lack of a cytotoxic T cell response against the host. Intriguingly, these conditions all seem to hold true in human SLE.[64]

Another possible mechanism that may involve chimerism in the pathogenesis in SLE is the occurrence of a host-versus-graft reaction, similar to rejection after solid organ transplantation. The host must recognise the chimeric cell as foreign for a host-versus-graft reaction to occur. During and after pregnancy, anti-paternal HLA antibodies have been found in up to 30% of women.[65] Several mechanisms prevent the immune system from the mother to react against the paternal antigens during pregnancy.[66] However, when these tolerance mechanisms are no longer in effect after delivery, it is possible that the immune system from the mother reacts against the fetal cells that may have been incorporated into various tissues by that time. Because a host-versus-graft reaction would expectedly result in the elimination of chimeric cells, the disease manifestations would be localised and limited. This may parallel the clinical situation of an SLE patient who experiences a relatively short and limited course of the disease. If however, the removal of chimeric cells fails, e.g. due to an inadequate response of cytotoxic T cells or NK cells, the chimeric cells may be able to continuously stimulate the immune system leading to persistent inflammation resembling autoimmune disease.[64] Apart from a direct response to the chimeric cells, the immune response can also be sustained by molecular mimicry. In this case, the chimeric cell induces a host-versus-graft reaction, which in itself is self-limited, but because of cross-reactivity between antigens on chimeric cells and self-antigens of the host, autoimmunity occurs.[64]

In contrast to the proposed effects of chimerism mentioned above, chimeric cells may also be involved in tissue repair. As previously mentioned, pregnancy may result in the acquisition of fetal chimeric cells with the capacity for multilineage differentiation and tissue repair. Studies in mice have demonstrated that fetal chimeric cells migrate to sites of maternal injury.[67, 68] In a human autopsy study, Kremer Hovinga *et al.*[57] found significantly more microchimerism in organs from women with SLE that showed either SLE-related or non-SLE-related injury, than in organs from women with SLE without injury and in uninjured organs from controls. No difference in the occurrence of microchimerism was found between uninjured organs from SLE patients and uninjured organs from controls, indicating that SLE patients did not have a higher "background" level of chimerism. Because chimeric cells also seem to occur in tissues without apparent injury,[69, 70] normal tissue maintenance may also be responsible for the occurrence of microchimerism in tissues.

Other than having either a pathogenic or a beneficial role, it is also possible that chimeric cells are innocent bystanders that do not react with the immune system of the host. If one assumes that chimeric cells are distributed equally across tissues, and the cell density increases in inflamed tissue, more chimeric cells would reasonably be found in injured tissues. This hypothesis was investigated in an autopsy study by Kremer Hovinga *et al.*[57] Although a tendency towards an increased occurrence of microchimerism was found when an inflammatory infiltrate was present, the occurrence of microchimerism could not be solely explained by an influx of inflammatory cells.

Because pregnancy is very common and SLE is relatively rare, it is likely that only certain subsets of chimeric cells are pathogenic. This may depend on the phenotype of the chimeric cells and HLA relationships between the chimeric cell and the host. Also, microchimerism may not be only beneficial or pathogenic, but rather a combination of both depending on the circumstances. Probably, only some chimeric cells with an immunocompetent phenotype have pathogenic potential, whereas others, such as CD34+ cells found in pregnancy, may be innocent bystanders. In the setting of fetomaternal cell trafficking, it is important to realise that the proportion of cell phenotypes is different between the mother and fetus. The different subpopulations of chimeric cells in the mother and fetus may explain why many autoimmune diseases are less common in neonates and young children than in adults, but further research is warranted.

Pathogenesis

As previously mentioned, pathogenic autoantibodies against nuclear components currently form an important pillar in the classification of SLE and may be of pivotal importance in making the diagnosis. Many clinical manifestations of SLE are mediated by circulating immune complexes that form when autoantibodies bind nuclear antigens and deposit in various tissues or by direct binding of autoantibodies to antigens on resident cell surfaces or extracellular components in various organs. Both situations result in the attraction and activation of infiltrating leukocytes resulting in the release of various inflammatory mediators, including cytokines, growth factors, vasoactive substances, complement, and coagulation factors. Receptors for the Fc portion of deposited immunoglobulin are present on many immune cells. Activation of Fc receptors induces a number of responses, including Fc receptor-mediated phagocytosis and antibody-dependent cell-mediated cytotoxicity.[71] However, the complement system appears to be a key mediator of immunoglobulin-induced tissue injury in SLE.

The complement system, an essential component of the innate immune system, is a complex cascade of activation of plasma and membrane-bound proteins that are divided according to their respective surface recognition patterns into three major pathways: the classical pathway, the lectin pathway, and the alternative pathway.[72] Immune complexes formed by autoantibodies and antigens lead to the activation of the classical pathway. Activation of the classical pathway is initiated by the binding of complement factor C1q and

activation of the C1 complex, leading to the formation of C3 convertase and the cleavage of complement component C3. Cleavage of C3, the most abundant serum complement protein, results in the release of the chemotactic factor C3a and covalent attachment of C3b to host cells, which is also important for the amplification of the cascade through the alternative pathway and for continued activation of the complement system. In the process, the anaphylatoxins C3a and C5a attract neutrophils and monocytes by means of a strong chemotactic signal, which in turn propagate further tissue injury. Finally, the complement cascade results in the formation of the terminal membrane attack complex, C5b-9. C5b-9 causes cytolysis of the target cell by insertion in cell membranes in lytic quantities.

Importantly, while the activation of complement is apparently deleterious in the propagation of tissue injury, it was mentioned earlier that a deficiency of early complement components is associated with SLE itself. The latter is due to impaired clearance of immune complexes and/or apoptotic debris in patients lacking C1, C2, and C4, leading to a break of tolerance.[31] Clearly, the complement system plays a dual role in SLE.

Treatment and Prognosis

Given the clinical heterogeneity of SLE, treatment is highly variable depending on disease manifestations, disease activity and severity, comorbidities, and patient preferences. Generally, all patients with SLE receive treatment with the antimalarial drug hydroxychloroquine.[73] The benefits of hydroxychloroquine in the treatment of SLE are broad, including the amelioration of constitutional symptoms, musculoskeletal manifestations, and mucocutaneous manifestations; as well as the reduction of flare rates, thrombotic events, organ damage accrual, and mortality.[74] Additional therapy depends on the severity of specific manifestations. Patients with mild to moderate manifestations may be treated conservatively with nonsteroidal anti-inflammatory drugs, and/or (low-dose) corticosteroids. Occasionally, a steroid-sparing agent such as azathioprine may be indicated to maintain control of symptoms.[73] Patients with severe or life-threatening major organ involvement of SLE, such as lupus nephritis and neuropsychiatric lupus, usually require intensive cytotoxic immunosuppression to induce remission including high doses of intravenous corticosteroids in combination with other immunosuppressive agents, such as mycophenolate mofetil or cyclophosphamide. As will be discussed in more detail in **Part 2** of this introduction, these immunosuppressive agents are associated with severe and potentially lethal adverse effects,[75] and selection of patients for whom such treatment is indicated is therefore of utmost importance. The phase of intensive treatment is usually followed by a second phase of less toxic treatment to maintain remission.[73]

Partly attributed to these intensive schemes of treatment that were developed during the past decades, the prognosis of SLE has improved from less than 50% 5-year survival in 1955[76] to more than 90% 10-year survival in recent years.[77, 78] Several other factors may have contributed to this increased survival rate, including the improved capability to diagnose patients with (early) SLE, the increased recognition of patients with mild disease,

and the improved treatment of comorbid and secondary conditions, such as hypertension, infection, and renal failure.[79] Despite these improvements, mortality rates of patients with SLE are on average still two to five times higher than in the general population.[80] Given the heterogeneous manifestations and corresponding heterogeneous clinical course, the prognosis of individual patients largely depends on the presence of adverse prognostic factors, including lupus nephritis,[78] hypertension,[81] male sex, older age at presentation,[81] low socioeconomic status,[13] Afrocaribbean race,[13] presence of antiphospholipid antibodies,[82] and high overall disease activity.[81]

The decrease in overall mortality attributed partly to immunosuppressive therapy has resulted in a shift in causes of death among patients with SLE. Whereas in earlier days patients with SLE frequently died of causes relating to active SLE and infections, today the most frequent causes of death – including cardiovascular disease and malignancy – are not directly related to SLE. Immunosuppressive therapy may now be included as an adverse prognostic factor for these long-term outcomes. Various studies have indicated that besides SLE itself, also its treatment, especially corticosteroids and cytotoxic drugs, may play a role in these causes of death.[83-85] As mentioned earlier, these medications are given primarily to patients with major organ involvement of SLE – patients who are already at increased risk of adverse outcomes.

PART 2: FOCUS ON MAJOR ORGAN MANIFESTATIONS OF SLE

Lupus nephritis

Renal involvement due to lupus nephritis (LN) occurs in approximately one half of patients with SLE at some time in the course of their disease.[86] Clinical manifestations of LN range from asymptomatic urinary findings (microscopic haematuria or proteinuria) to the nephrotic syndrome and progressive renal dysfunction. Given the relatively frequent occurrence of LN in SLE and its potentially severe consequences, patients with SLE should undergo regular testing for renal involvement by evaluation of the urinary sediment, proteinuria, and serum creatinine. Elevated anti-dsDNA titres and low complement levels may indicate active SLE, and particularly LN, although the utility of serologic assessment differs among patients. Laboratory abnormalities indicating renal dysfunction in a patient with SLE require further diagnostic workup: they may indicate LN as well as an unrelated form of renal disease. A renal biopsy serves as a central diagnostic asset in LN. Not only can a renal biopsy confirm the diagnosis of LN and sometimes exclude other causes in a patient with clinical suspicion of LN, but it can also guide treatment decisions and predict outcome. The pathogenic mechanisms contributing to LN have been studied extensively and form the foundation of its tissue diagnosis. In turn, the tissue diagnosis guides the prognosis.

The kidney as a site of injury in SLE

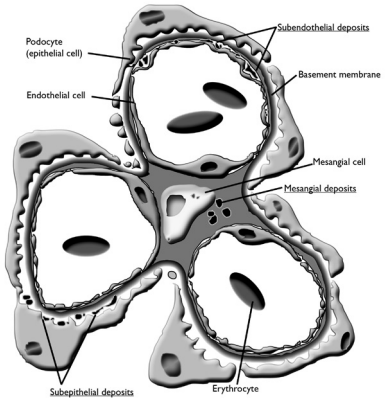
The anatomy and physiology of the kidney make it highly susceptible to inflammatory insults caused by autoantibodies. A number of factors contribute to the nonspecific trapping of immune complexes in the kidney.[87] First, immune complexes in the circulation are delivered at a high rate to the kidney because the kidney receives roughly 25% of the cardiac output. Second, intraglomerular pressure is higher than in other capillary beds, and more protein than usual may be forced across the glomerular capillary wall. Third, the glomerular capillaries provide a large and highly permeable surface through which immune complexes circulate. Lastly, the capillary walls comprise a negatively charged surface, facilitating the binding of positively charged macromolecules. Initiation of renal disease in SLE caused by preformed circulating immune complexes is likely due to the deposition of immune complexes in the mesangium or subendothelial spaces, since these complexes are too large to cross the capillary wall. Alternatively, immune deposits may form in situ when antibodies bind to intrinsic antigens in the kidney, such as extracellular matrix components or cell surface glycoproteins. In situ formation may also occur when soluble antigens become independently localised in the kidney due to charge interactions with anionic sites in the glomerular basement membrane. In the setting of LN, cationic histone parts of nucleosomes are bound to anionic glomerular basement membrane components such as heparan sulphate or collagen IV, resulting in binding of anti-dsDNA antibodies in situ.[88] Since the in situ formation of immune deposits depends on the location of the intrinsic antigen or the site where the extrinsic antigen is deposited, this type of immune complex deposition may occur in the mesangium, subendothelial, or subepithelial space. The reaction that these immune complexes elicit depends, in part, on the nature of the autoantibody (its ability to activate complement or to bind to Fc receptors).[89] Furthermore, depending on the site of immune complex deposition, different patterns of injury may be observed.

Immune deposits at sites accessible to the circulation, such as the subendothelial region or mesangium (**Figure 2**), tend to cause an inflammatory or proliferative form of glomerulonephritis. Mesangial deposits (**Figure 2**) result in activation of mesangial cells causing mesangial hypercellularity and production of extracellular matrix, generally resulting in microscopic haematuria and subnephrotic proteinuria along with a preserved glomerular filtration rate (GFR).[90,91] Subendothelial deposits may elicit inflammatory nephritis characterised by influx of leukocytes, endothelial cell injury, and endocapillary hypercellularity. This pattern is often associated with capillary wall destruction and varying degrees of crescent formation.[90] Because the mesangium is in direct continuity with the subendothelial space, various degrees of mesangial proliferation may also be observed – in its ultimate form recognised as mesangiocapillary or membranoproliferative nephritis. This pattern of injury may also be observed in the absence of immune complexes in the case of shear stress due to malignant hypertension, or thrombotic microangiopathy in SLE-associated antiphospholipid syndrome. The subendothelial pattern of injury is generally accompanied by a marked decrease in GFR, haematuria, and mild to moderate proteinuria.[91] In contrast, subepithelial deposits, secluded from inflammatory cells by the barrier formed by the glomerular base-

ment membrane, tend to produce a non-inflammatory form of complement-mediated podocyte injury that manifests mainly with (nephrotic-range) proteinuria.[90,91]

The detection of immune deposits by immunofluorescence at various glomerular locations as well as along tubular basement membranes and in vascular walls forms an essential diagnostic test in LN. The immune deposits predominantly contain polyclonal IgG, as well as C3, and in most instances C1q. A hallmark finding is the so-called "full house" staining pattern by immunofluorescence, defined as concurrent positivity for IgA, IgG, and IgM, as well as the complement components C3 and C1q.[91] The finding of this pattern is the result of nonspecific activation of autoreactive B cells resulting in the formation of autoantibodies with many specificities, as well as the activation of multiple pathways of complement. The immune deposits may also be detected by electron microscopy, giving an electron-dense appearance varying in size and distribution.

Figure 2 Localisations of immune complexes in the glomerulus in lupus nephritis.



Sequelae of immune complex-mediated injury in the kidney

Damage to renal parenchymal cells triggers healing responses that contribute to renal pathology. Focal necrosis in the glomerular tuft may be followed by migration of parietal epithelial cells towards the visceral epithelial cells forming cellular bridges and their subsequent production of extracellular matrix, contributing to focal segmental glomerulosclerosis, which may eventually progress to global glomerulosclerosis.[92] In addition, cellular crescent formation may also result from activation of parietal epithelial cells that fill Bowman's space by proliferation.[93, 94] This process can be triggered by breaks in the glomerular basement membrane that allow plasma to leak into Bowman's space.[95] In later stages, the parietal epithelial cells initiate a process of extensive matrix production, creating a "honeycomb" matrix in Bowman's space that turns cellular crescents into fibrocellular crescents and eventually fibrous crescents and glomerulosclerosis.

Histopathologic classification of LN

Since the introduction of the renal biopsy in the 1950s, a number of efforts have been made to classify LN based on the knowledge about immune complex-mediated pathogenesis and evidence indicating the clinical significance of various lesions. A histopathologic classification serves to implement the histopathologic diagnosis with prognostic information in clinical practice, as well as providing a means for communication between pathologists and clinicians and allowing risk stratification of patients included in clinical intervention studies. Following the World Health Organisation (WHO) classifications,[96, 97] the International Society of Nephrology and Renal Pathology Society (ISN/RPS) working group construed a new classification of LN in 2003, which has presently gained world-wide acceptance.[90] The ISN/RPS 2003 classification of LN categorises the spectrum of lesions occurring in LN into discrete entities, consisting of six classes based on a mesangial (classes I/II), proliferative (classes III/IV), membranous (class V), or a globally sclerotic (class VI) pattern of injury (**Table 3**).

Treatment and prognosis

LN is associated with considerable morbidity and mortality. The cumulative 5-year survival of LN has improved from 50% in the 1960s to 80% in the 1990s, reflecting the implementation of immunosuppressive therapy.[98] Even though immunosuppressive treatment is clearly beneficial for some patients with LN, patients eligible for such therapy should be selected with great caution due to severe and potentially lethal adverse effects.[75] Many clinical trials on therapy in LN have been published over the past 40 years. The conclusions from these trials have been incorporated in a set of currently employed national and international treatment guidelines for LN.[99-103] Because of a poor correlation between clinical and biopsy findings,[104, 105] and because early diagnosis and treatment have been shown to improve outcomes in LN,[106-108] the threshold for a renal biopsy is set relatively low. Thus, the guidelines uniformly recommend a renal biopsy in patients with SLE and any suspicion of renal involvement. Specifically, this is meant to indicate a decrease in renal function, reproducible proteinuria (>500 mg/24h), and/or the presence of an active urinary sediment. Importantly,

guidelines base therapeutic decisions in LN solely on the histopathologic diagnosis of LN class according to the ISN/RPS classification.[90]

Table 3 ISN/RPS 2003 classification of lupus nephritis.[90]

Class I	**Minimal mesangial LN** Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence
Class II	**Mesangial proliferative LN** Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits A few isolated subepithelial or subendothelial deposits may be visible by immunofluorescence or electron microscopy, but not by light microscopy
Class III	**Focal proliferative LN** Active or inactive focal, segmental or global endo- or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations
Class IV	**Diffuse proliferative LN** Active or inactive diffuse, segmental or global endo- or extracapillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) LN when ≥50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) LN when ≥50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits but with little or no glomerular proliferation.
Class V	**Membranous LN** Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations Class V LN may occur in combination with class III or IV in which case both will be diagnosed Class V LN may show advanced sclerosis
Class VI	**Advanced sclerotic LN** ≥90% of glomeruli globally sclerosed without residual activity

LN, lupus nephritis.

Treatment for class II

Due to lack of evidence, guidelines for LN are inconsistent with regard to therapy for class II LN.[109] There is consensus that proteinuria should be controlled with renin-angiotensin-aldosterone system (RAAS) inhibitors. As to immunosuppressive therapy, it is generally accepted that class II LN does not require such therapy, although corticosteroids may be indicated in patients with proteinuria over 1 g/24h, particularly in the presence of glomerular haematuria.[99]

Treatment for classes III and IV

Based on evidence from a number of landmark randomised controlled trials (RCTs), tre-

atment guidelines for LN uniformly recommend immunosuppressive therapy for patients with class III or IV LN.[109] A series of RCTs in the 1970s indicated that long-term use of a combination of corticosteroids and high-dose intravenous cyclophosphamide (0.5–1 g/m^2 monthly for 6 months) was superior to steroids alone to prevent renal impairment.[110-112] Based on this finding, the so-called "NIH regimen" became the standard of care for induction of remission of classes III and IV LN for two decades, despite its many side effects including a high rate of severe infections and premature ovarian failure. More recently, two different approaches for induction of remission of classes III and IV LN have been investigated. First, the "Euro-Lupus" regimen was proposed to potentially decrease the burden of cytoxic immunosuppression by lowering doses of intravenous cyclophosphamide for induction of remission (500 mg fortnightly for 3 months). In the Euro-Lupus trial, this regimen was shown to achieve results comparable with high-dose intravenous cyclophosphamide.[113] Second, mycophenolate mofetil (MMF) was shown to be at least as efficacious as intravenous cyclophosphamide to induce a satisfactory renal remission at 6 months in several studies,[114, 115] with the advantage that this drug conveys a lower risk of premature ovarian failure.[116] On account of these studies, current guidelines recommend intravenous cyclophosphamide (either Euro-Lupus or NIH regimen) or MMF (2–3 g total daily dose) in combination with oral corticosteroids with or without three pulses of intravenous methylprednisolone at the start of induction treatment. In the maintenance phase of treatment, MMF (1–2 g/day) or azathioprine (1.5–2.5 mg/kg/day) is recommended, supported by low-dose oral corticosteroids.[99-103]

Treatment for class V

Immunosuppressive treatment for class V LN is generally not recommended, unless a patient has nephrotic-range proteinuria (>3 g/24h).[109] With sub-nephrotic proteinuria, anti-proteinuric treatment with RAAS inhibitors is indicated. Evidence on the efficacy of immunosuppressive treatment for class V LN is limited; therefore the recommended management of class V LN differs between guidelines.[99-103] However, there is an agreement that corticosteroids should be included in the immunosuppressive regimen for patients with nephrotic-range proteinuria. In addition, MMF,[73, 100, 101, 103] cyclophosphamide,[101, 103] azathioprine,[101, 103] rituximab,[101, 103] or calcineurin inhibitors[101, 103] are generally advised.

Neuropsychiatric lupus

Neuropsychiatric involvement in SLE (NP-SLE) is observed in 10-80% of patients.[117-121] The manifestations of NP-SLE represent a spectrum of disorders, both with focal and diffuse symptoms. Although headache and mood disorders are the most frequent neuropsychiatric complaints in patients with SLE, seizure disorder, cerebrovascular disease, acute confusional state, and neuropathy are the most common neuropsychiatric syndromes attributed to SLE. A major difficulty in the diagnosis of NP-SLE is the lack of clear diagnostic definitions, which is caused by a lack of pathognomonic features, inadequacy of diagnostic tools, and a vast heterogeneity of clinical disorders. In contrast to LN, where knowledge about pathogenic mechanisms is central in establishing a tissue diagnosis and conjoint prognosis, little is known about the pathogenesis of NP-SLE. Due to the impracticability of performing a brain biopsy, histopathologic studies elucidating pathogenic mechanisms are limited and a tissue diagnosis is generally not possible. Therefore, the clinical diagnosis of NP-SLE is founded on clinical and neuropsychological assessment, aided by laboratory analyses of blood and cerebrospinal fluid (to exclude central nervous system infection), electroencephalographic analysis (to diagnose a seizure disorder), nerve conduction studies (to diagnose peripheral neuropathy) and magnetic resonance imaging (MRI).[122] MRI is a central tool in the diagnosis of NP-SLE-related injury, since this modality has the capacity to identify brain infarctions as well as confounding disorders such as space-occupying lesions, infectious meningitis or brain abscesses. Unfortunately, there is not one MRI finding or pattern that is diagnostic or specific for NP-SLE. In the presence of clinical symptoms, MRI often shows no abnormalities or unspecific abnormalities such as small white matter hyperintensities,[123] known as the clinicoradiological paradox. Furthermore, MRI alone cannot distinguish between thromboembolic and inflammatory insults in many patients.[124]

In 1999 "The ACR Nomenclature and Case Definitions for Neuropsychiatric Lupus Syndromes" was published, serving as a guide for clinicians and researchers to identify individual NP-SLE disorders (**Table 4**).[125] Because NP-SLE remains a condition that is diagnosed *per exclusionem*, the ACR nomenclature also defines several conditions that must be excluded before it can be established that a neuropsychiatric manifestation is the result of the disease itself (primary NP-SLE). Possibly 40% of all neuropsychiatric disorders in SLE patients are the consequence of secondary conditions related to SLE, such as metabolic disturbances attributed to LN, hypertension, and side effects of medications (secondary NP-SLE).

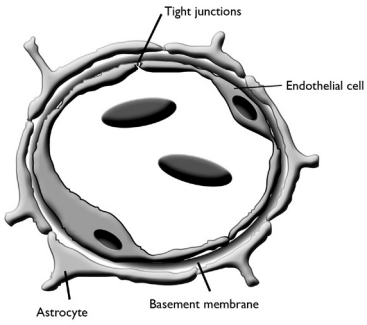
Table 4 Neuropsychiatric syndromes that may occur in SLE as defined by the ACR.[125]

Central nervous system	Peripheral nervous system
1. Aseptic meningitis	13. Guillain-Barré syndrome
2. Cerebrovascular disease	14. Autonomic disorder
3. Demyelinating syndrome	15. Mononeuropathy single/multiplex
4. Headache	16. Myasthenia gravis
5. Movement disorder (chorea)	17. Cranial neuropathy
6. Myelopathy	18. Plexopathy
7. Seizure disorders	19. Polyneuropathy
8. Acute confusional state	
9. Anxiety disorder	
10. Cognitive dysfunction	
11. Mood disorder	
12. Psychosis	

The brain as a site of injury in SLE

The brain represents a unique environment in the study of immune complex-mediated injury in SLE.[126] Since the brain has limited capacity for repair and regeneration of neurons, the immunologic barrier in the brain helps to minimise damage. This immunologic barrier is referred to as the blood-brain barrier, and consists of polarised endothelial cells connected by tight junctions, further supported by foot processes of astrocytes (**Figure 3**). This barrier prevents entry of cells and macromolecules including immunoglobulins into the central nervous system. Under normal circumstances, there is little T cell trafficking into the central nervous system and negligible production of antibodies by B cells in the brain. The attenuated cellular response in the brain limits harmless bystander injury of neurologic tissue that would occur during a regular immune response. Studies on the pathogenic mechanisms contributing to tissue injury in NP-SLE in this unique immunologic environment are limited.

Figure 3 The blood-brain barrier.



On the basis of neuropathological findings in a subset of patients with NP-SLE, neuropsychiatric involvement in SLE seems to be characterised by a vascular, thrombo-ischemic pathogenic mechanism. Microvascular occlusions with hyaline or platelet microthrombi, microinfarctions and small vessel vasculopathy are the most common findings in all neuropathological studies that have been performed to date.[127, 128] Particularly vasculopathy appears to be a very common finding in NP-SLE, defined as endothelial proliferation, hyalinisation, and thickening of the vessel wall. The alterations in the blood vessel wall recognised as vasculopathy are not accompanied by inflammatory cells in the vessel wall, and therefore cannot be classified as vasculitis. Less commonly, macroscopic infarction or haemorrhage may be observed, the former occasionally due to an embolism from Libman-Sacks endocarditis, or due to the consequences of antiphospholipid antibodies. Unlike these destructive macrovascular changes, the microvascular changes are poorly correlated with central nervous system disease, and these changes may be very prominent in cases with minimal or no neurologic symptoms.[127] Intriguingly, true vasculitis appeared to be a rare finding in NP-SLE in various studies.[129, 130] The pathophysiology of the widespread microvascular injury in NP-SLE is unknown. Clinical syndromes thought to be related to thromboischemic pathology include stroke and cognitive dysfunction.[131]

In apparent contrast with the thromboischemic pathogenic mechanism just described, a mechanism involving inflammation and neurotoxic autoantibodies has also been implicated in NP-SLE. A number of reports have noted immunologic abnormalities including elevated levels of anti-dsDNA antibodies, oligoclonal banding, immune complexes, interleukin-6, and markers of B-cell activation in the cerebrospinal fluid of patients with NP-SLE.[132-134] Moreover, a number of autoantibodies have been associated with different aspects of NP-SLE. To date, 11 autoantibodies directed to intrinsic brain components and nine autoantibodies that are also found in general SLE populations have been described in NP-SLE.[135] However, none of these autoantibodies appear to be specific for any NP-SLE manifestation. Clinical syndromes that are presumed to relate to inflammatory autoimmune pathology are diffuse neuropsychiatric manifestations including psychosis and acute confusional state.[131]

Immune complex deposition in the small cerebral vessels of SLE patients has never been demonstrated. Since complement-mediated injury is a key event in many of the other organ manifestations of SLE, this mechanism could play a similar role in NP-SLE. In the setting of NP-SLE, one hypothesis is that circulating immune complexes may activate complement via binding of C1q and activation of the classical pathway. Studies have identified a number of possible mechanisms that may contribute to subsequent tissue injury in the brain. For instance, C5a can induce heparin-sulphate release from endothelial cell membranes, promoting endothelial proliferation and upregulation of e-selectin and vascular cell adhesion molecule (VCAM).[136] Also, the complement system, closely related to the coagulation cascade, may mediate secretion of von Willebrand factor and Tissue Factor expression in response to C5b-9-induced endothelial injury creating a procoagulant

state.[137, 138] Studies in mice suggest that complement may also amplify thromboischemic damage: in neonatal mice, the infarcted area after clipping of a cerebral artery was over three times smaller in C1q-deficient mice compared with wild type mice.[139] In this thesis, the relationship between complement and thromboischemic injury NP-SLE was explored further.

Treatment and prognosis

A major difficulty in the treatment of NP-SLE is the unavailability of targeted therapy due to the uncertainty about pathogenic mechanisms. Treatment involves the management of comorbidities contributing to the neuropsychiatric event, controlling of symptoms, as well as more specific interventions including immunosuppressive and anticoagulation therapy. Corticosteroids and cytotoxic immunosuppressive therapy are indicated when NP-SLE is thought to reflect an inflammatory process (optic neuritis, transverse myelitis, peripheral neuropathy, refractory seizures, psychosis, and acute confusional state) and in the presence of generalised SLE activity. Antiplatelet/anticoagulation therapy is indicated when manifestations are related to antiphospholipid antibodies, particularly thrombotic cardiovascular disease.[122] The differentiation between an inflammatory or underlying thromboischemic pathogenic mechanism may not be feasible and in some patients both mechanisms may be operant.

The reported prognosis of NP-SLE is highly variable: several studies have documented an increased mortality in patients with NP-SLE compared to SLE without neuropsychiatric symptoms,[98, 140-142] whereas others have not[120, 143, 144]

PART 3: THIS THESIS

The interpretation of Hippocrates' quote in the prologue of this thesis in the setting of SLE uncovers a number of challenges faced by the physician when treating SLE patients. First, "telling the antecedents" and "knowing the present" in SLE – essentially making a diagnosis based on common aetiology, pathogenesis, or symptoms – is challenged by the multifactorial aetiology, the multiple routes of pathogenesis, and the vast diversity of clinical manifestations of this disease. Second, "foretelling the future" may be challenged by the same factors complicating diagnosis. As became clear in this introduction, knowledge about pathogenic mechanisms plays a crucial role in the diagnosis and prognosis of SLE. Two contrasting examples with regard to such knowledge – LN and NP-SLE – clearly demonstrate this. In LN, the pathogenesis of immune complex deposition is relatively well studied and forms the basis of tissue diagnosis, prognosis, and treatment decisions. In contrast, the pathogenesis of NP-SLE is poorly studied, and diagnostic tools, prognostic, and therapeutic indicators are relatively limited. Also, clinical heterogeneity between patients may greatly affect outcomes, and thereby complicate the ability to estimate the prognosis for an individual patient. Third, the objectives "to do good or to do no harm" reflect the ever-challenging trade-off between therapeutic benefits of intensive immunosuppressive therapy for life-threatening manifestations of SLE, and the concurrently harmful and possibly life-threatening adverse effects of these therapies. In this thesis, a number of these challenges were investigated.

In the first part of this thesis, challenges in diagnosing SLE were investigated in the setting of patients with nephritis showing full house glomerular immune deposits. The full house pattern by immunofluorescence is regarded as very characteristic of SLE, and it is unknown whether this finding in a patient with absent systemic signs or symptoms of SLE warrants its clinical distinction from LN and SLE. In **chapter 2**, the SLICC classification criteria were validated in a cohort of patients with full house glomerular immune deposits, aiming to resolve whether SLE classification criteria may be applied to patients from the nephrology clinic with renal involvement suggestive of LN. **Chapter 3** is focused on the distinction between patients with lupus-like renal involvement with full house glomerular deposits in the setting of clinically confirmed LN (lupus full house nephropathy) and such patients who do not have SLE (non-lupus full house nephropathy). In this chapter, a special focus lies on the clinical, histopathologic, and prognostic differentiation between lupus and non-lupus full house nephropathy, aiming to clarify whether lupus and non-lupus full house nephropathy should be regarded as clinically distinct entities.

In the second part of this thesis, the aforementioned challenges were investigated in two of the most life threatening visceral manifestations of SLE: LN and NP-SLE. In **chapter 4**, patients with class III and IV LN were investigated who did not receive cytotoxic immunosuppression. In this study, the natural history of classes III and IV LN was assessed,

aiming to identify a subgroup of patients with a favourable prognosis eligible for treatment without cytotoxic immunosuppression. In **chapter 5**, the goal was to advance patient-tailored care for patients with LN by means of the evidence-based identification of clinical and histopathologic prognostic indicators of renal outcome in classes I–V LN. In the setting of NP-SLE, complement activation as a pathogenic mechanism was investigated to provide a possible link between thromboischemic injury in NP-SLE and autoantibody-mediated injury characteristic of SLE. In **chapter 6**, the presence of classical complement deposition in cerebral tissue of patients with SLE was examined, and the association between complement and thromboischemic cerebral injury was assessed.

In the third part of this thesis, chimerism as a potential aetiologic factor of SLE was studied. To substantiate ongoing research relating microchimerism to autoimmune disease, the occurrence of tissue microchimerism during human pregnancy was investigated in **chapter 7**. In **chapter 8**, the origin and amount of microchimerism in peripheral blood of women with SLE and controls was studied.

REFERENCES

1. Newman DH. Hippocrates' Shadow. New York: Scribner; 2009.
2. Liu Z, Davidson A. Taming lupus-a new understanding of pathogenesis is leading to clinical advances. Nat Med. 2012; 18(6): 871-82.
3. Blotzer JW. Systemic lupus erythematosus I: historical aspects. Md State Med J. 1983; 32(6): 439-41.
4. Smith CD, Cyr M. The history of lupus erythematosus. From Hippocrates to Osler. Rheum Dis Clin North Am. 1988; 14(1): 1-14.
5. Hebra F. On Diseases of the Skin including the Exanthemata. London: The New Sydenham Society; 1866.
6. Kaposi M. Neue Beitrage zur Keantiss des lupus erythematosus. Arch Dermatol Syphilol. 1872; 4(36).
7. Libmann E, Sacks B. A hitherto undescribed form of volvular and mural endocarditis. Arch Intern Med. 1924; (33): 701.
8. Baehr G, Klemperer P, Schifrin A. A diffuse disease of the peripheral circulation (usually associated with lupus erythematosus and endocarditis). Am J Med. 1952; 13(5): 591-6.
9. Scofield RH, Oates J. The place of William Osler in the description of systemic lupus erythematosus. Am J Med Sci. 2009; 338(5): 409-12.
10. Hargraves MM, Richmond H, Morton R. Presentation of two bone marrow elements; the tart cell and the L.E. cell. Proc Staff Meet Mayo Clin. 1948; 23(2): 25-8.
11. Miescher P, Fauconnet M. [Absorption of L. E. factor by isolated cell nuclei]. Experientia. 1954; 10(6): 252-3.
12. Friou GJ, Finch SC, Detre KD. Interaction of nuclei and globulin from lupus erythematosis serum demonstrated with fluorescent antibody. J Immunol. 1958; 80(4): 324-9.
13. Pons-Estel GJ, Alarcon GS, Scofield L, Reinlib L, Cooper GS. Understanding the epidemiology and progression of systemic lupus erythematosus. Semin Arthritis Rheum. 2010; 39(4): 257-68.
14. Lahita RG. The role of sex hormones in systemic lupus erythematosus. Curr Opin Rheumatol. 1999; 11(5): 352-6.
15. Chakravarty EF, Bush TM, Manzi S, Clarke AE, Ward MM. Prevalence of adult systemic lupus erythematosus in California and Pennsylvania in 2000: estimates obtained using hospitalization data. Arthritis Rheum. 2007; 56(6): 2092-4.
16. Von Feldt JM. Systemic lupus erythematosus. Recognizing its various presentations. Postgrad Med. 1995; 97(4): 79-83.
17. Aggarwal R, Ringold S, Khanna D, Neogi T, Johnson SR, Miller A, *et al.* Distinctions Between Diagnostic and Classification Criteria? Arthritis Care Res. 2015; 67(7): 891-7.
18. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997; 40(9): 1725.
19. Petri M, Orbai AM, Alarcon GS, Gordon C, Merrill JT, Fortin PR, *et al.* Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012; 64(8): 2677-86.
20. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1982; 25(11): 1271-7.
21. Petri M, Magder L. Classification criteria for systemic lupus erythematosus: a review. Lupus. 2004; 13(11): 829-37.
22. Lom-Orta H, Alarcon-Segovia D, Diaz-Jouanen E. Systemic lupus erythematosus. Differences between patients who do, and who do not, fulfill classification criteria at the time of diagnosis. J Rheumatol. 1980; 7(6): 831-7.

23. Calvo-Alen J, Alarcon GS, Burgard SL, Burst N, Bartolucci AA, Williams HJ. Systemic lupus erythematosus: predictors of its occurrence among a cohort of patients with early undifferentiated connective tissue disease: multivariate analyses and identification of risk factors. J Rheumatol. 1996; 23(3): 469-75.
24. Molin S, Ruzicka T, Prinz JC. Borreliosis mimicking lupus-like syndrome during infliximab treatment. Clin Exp Dermatol. 2010; 35(6): 631-3.
25. Sakkas LI, Boulbou M, Kyriakou D, Makri I, Sinani C, Germenis A, *et al.* Immunological features of visceral leishmaniasis may mimic systemic lupus erythematosus. Clin Biochem. 2008; 41(1-2): 65-8.
26. Nesher G, Osborn TG, Moore TL. Parvovirus infection mimicking systemic lupus erythematosus. Semin Arthritis Rheum. 1995; 24(5): 297-303.
27. Saravana S, James DW, Abourawi F, Gupta PC, Samyukta B. HIV infection mimicking SLE. Clin Rheumatol. 2004; 23(6): 562-3.
28. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, *et al.* Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med. 2003; 349(16): 1526-33.
29. Mistry P, Kaplan MJ. Cell death in the pathogenesis of systemic lupus erythematosus and lupus nephritis. Clin Immunol. 2016; doi: 10.1016/j.clim.
30. Barbhaiya M, Costenbader KH. Ultraviolet radiation and systemic lupus erythematosus. Lupus. 2014; 23(6): 588-95.
31. Truedsson L, Bengtsson AA, Sturfelt G. Complement deficiencies and systemic lupus erythematosus. Autoimmunity. 2007; 40(8): 560-6.
32. Walport MJ. Lupus, DNase and defective disposal of cellular debris. Nat Genet. 2000; 25(2): 135-6.
33. Rekvig OP, Van der Vlag J. The pathogenesis and diagnosis of systemic lupus erythematosus: still not resolved. Semin Immunopathol. 2014; 36(3): 301-11.
34. Block SR, Winfield JB, Lockshin MD, D'Angelo WA, Christian CL. Studies of twins with systemic lupus erythematosus. A review of the literature and presentation of 12 additional sets. Am J Med. 1975; 59(4): 533-52.
35. Kuo CF, Grainge MJ, Valdes AM, See LC, Luo SF, Yu KH, *et al.* Familial Aggregation of Systemic Lupus Erythematosus and Coaggregation of Autoimmune Diseases in Affected Families. JAMA Intern Med. 2015; 175(9): 1518-26.
36. Boackle SA. Advances in lupus genetics. Curr Opin Rheumatol. 2013; 25(5): 561-8.
37. Walport MJ, Black CM, Batchelor JR. The immunogenetics of SLE. Clin Rheum Dis. 1982; 8(1): 3-21.
38. Erkan D, Sammaritano L. New insights into pregnancy-related complications in systemic lupus erythematosus. Curr Rheumatol Rep. 2003; 5(5): 357-63.
39. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proc Natl Acad Sci U S A. 1996; 93(2): 705-8.
40. Khosrotehrani K, Johnson KL, Lau J, Dupuy A, Cha DH, Bianchi DW. The influence of fetal loss on the presence of fetal cell microchimerism: a systematic review. Arthritis Rheum. 2003; 48(11): 3237-41.
41. Bianchi DW, Farina A, Weber W, Delli-Bovi LC, Deriso M, Williams JM, *et al.* Significant fetal-maternal hemorrhage after termination of pregnancy: implications for development of fetal cell microchimerism. Am J Obstet Gynecol. 2001; 184(4): 703-6.
42. Sutton MS, Theard MA, Bhatia SJ, Plappert T, Saltzman DH, Doubilet P. Changes in placental blood flow in the normal human fetus with gestational age. Pediatr Res. 1990; 28(4): 383-7.
43. Moore KL. The developing human. Clinically oriented embryology. Philidelphia, PA, USA: Saunders Company; 2002.

44. Ariga H, Ohto H, Busch MP, Imamura S, Watson R, Reed W, *et al.* Kinetics of fetal cellular and cell-free DNA in the maternal circulation during and after pregnancy: implications for noninvasive prenatal diagnosis. Transfusion. 2001; 41(12): 1524-30.
45. Herzenberg LA, Bianchi DW, Schroder J, Cann HM, Iverson GM. Fetal cells in the blood of pregnant women: detection and enrichment by fluorescence-activated cell sorting. Proc Natl Acad Sci U S A. 1979; 76(3): 1453-5.
46. Little MT, Langlois S, Wilson RD, Lansdorp PM. Frequency of fetal cells in sorted subpopulations of nucleated erythroid and CD34+ hematopoietic progenitor cells from maternal peripheral blood. Blood. 1997; 89(7): 2347-58.
47. Bianchi DW, Flint AF, Pizzimenti MF, Knoll JH, Latt SA. Isolation of fetal DNA from nucleated erythrocytes in maternal blood. Proc Natl Acad Sci U S A. 1990; 87(9): 3279-83.
48. Mueller UW, Hawes CS, Wright AE, Petropoulos A, DeBoni E, Firgaira FA, *et al.* Isolation of fetal trophoblast cells from peripheral blood of pregnant women. Lancet. 1990; 336(8709): 197-200.
49. Douglas GW, Thomas L, Carr M, Cullen NM, Morris R. Trophoblast in the circulating blood during pregnancy. Am J Obstet Gynecol. 1959; 78: 960-73.
50. Walknowska J, Conte FA, Grumbach MM. Practical and theoretical implications of fetal-maternal lymphocyte transfer. Lancet. 1969; 1(7606): 1119-22.
51. Wessman M, Ylinen K, Knuutila S. Fetal granulocytes in maternal venous blood detected by in situ hybridization. Prenat Diagn. 1992; 12(12): 993-1000.
52. Khosrotehrani K, Johnson KL, Cha DH, Salomon RN, Bianchi DW. Transfer of fetal cells with multilineage potential to maternal tissue. J Am Med Assoc. 2004; 292(1): 75-80.
53. Stevens AM, Hermes HM, Rutledge JC, Buyon JP, Nelson JL. Myocardial-tissue-specific phenotype of maternal microchimerism in neonatal lupus congenital heart block. Lancet. 2003; 362(9396): 1617-23.
54. Artlett CM, Ramos R, Jiminez SA, Patterson K, Miller FW, Rider LG. Chimeric cells of maternal origin in juvenile idiopathic inflammatory myopathies. Childhood Myositis Heterogeneity Collaborative Group. Lancet. 2000; 356(9248): 2155-6.
55. Reed AM, Picornell YJ, Harwood A, Kredich DW. Chimerism in children with juvenile dermatomyositis. Lancet. 2000; 356(9248): 2156-7.
56. Kremer Hovinga I, Koopmans M, Baelde HJ, van der Wal AM, Sijpkens YW, de Heer E, *et al.* Chimerism occurs twice as often in lupus nephritis as in normal kidneys. Arthritis Rheum. 2006; 54(9): 2944-50.
57. Kremer Hovinga I, Koopmans M, Baelde HJ, de Heer E, Bruijn JA, Bajema IM. Tissue chimerism in systemic lupus erythematosus is related to injury. Ann Rheum Dis. 2007; 66(12): 1568-73.
58. Florim GM, Caldas HC, de Melo JC, Baptista MA, Fernandes IM, Savoldi-Barbosa M, *et al.* Fetal microchimerism in kidney biopsies of lupus nephritis patients may be associated with a beneficial effect. Arthritis Res Ther. 2015; 17(1): 101.
59. Kekow M, Barleben M, Drynda S, Jakubiczka S, Kekow J, Brune T. Long-term persistence and effects of fetal microchimerisms on disease onset and status in a cohort of women with rheumatoid arthritis and systemic lupus erythematosus. BMC Musculoskelet Disord. 2013; 14: 325.
60. da Silva Florim GM, Caldas HC, Pavarino EC, Bertollo EM, Fernandes IM, Abbud-Filho M. Variables associated to fetal microchimerism in systemic lupus erythematosus patients. Clin Rheumatol. 2016; 35(1): 107-11.
61. Gannage M, Amoura Z, Lantz O, Piette JC, Caillat-Zucman S. Feto-maternal microchimerism in connective tissue diseases. Eur J Immunol. 2002; 32(12): 3405-13.
62. Mosca M, Curcio M, Lapi S, Valentini G, D'Angelo S, Rizzo G, *et al.* Correlations of Y chromosome microchimerism with disease activity in patients with SLE: analysis of preliminary data. Ann Rheum Dis. 2003; 62(7): 651-4.

63. Via CS, Shearer GM. T-cell interactions in autoimmunity: insights from a murine model of graft-versus-host disease. Immunol Today. 1988; 9(7-8): 207-13.
64. Kremer Hovinga I, Koopmans M, de Heer E, Bruijn JA, Bajema IM. Chimerism in systemic lupus erythematosus--three hypotheses. Rheumatology (Oxford). 2007; 46(2): 200-8.
65. Regan L, Braude PR, Hill DP. A prospective study of the incidence, time of appearance and significance of anti-paternal lymphocytotoxic antibodies in human pregnancy. Hum Reprod. 1991; 6(2): 294-8.
66. Alijotas-Reig J, Llurba E, Gris JM. Potentiating maternal immune tolerance in pregnancy: a new challenging role for regulatory T cells. Placenta. 2014; 35(4): 241-8.
67. Wang Y, Iwatani H, Ito T, Horimoto N, Yamato M, Matsui I, *et al.* Fetal cells in mother rats contribute to the remodeling of liver and kidney after injury. Biochem Biophys Res Commun. 2004; 325(3): 961-7.
68. Tan XW, Liao H, Sun L, Okabe M, Xiao ZC, Dawe GS. Fetal microchimerism in the maternal mouse brain: a novel population of fetal progenitor or stem cells able to cross the blood-brain barrier? Stem Cells. 2005; 23(10): 1443-52.
69. Koopmans M, Kremer Hovinga I, Baelde HJ, Fernandes RJ, de Heer E, Bruijn JA, *et al.* Chimerism in kidneys, livers and hearts of normal women: implications for transplantation studies. Am J Transplant. 2005; 5(6): 1495-502.
70. Koopmans M, Kremer Hovinga I, Baelde HJ, Harvey MS, de Heer E, Bruijn JA, *et al.* Chimerism occurs in thyroid, lung, skin and lymph nodes of women with sons. J Reprod Immunol. 2008; 78(1): 68-75.
71. Niederer HA, Clatworthy MR, Willcocks LC, Smith KG. FcgammaRIIB, FcgammaRIIIB, and systemic lupus erythematosus. Ann N Y Acad Sci. 2010; 1183: 69-88.
72. Merle NS, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement System Part I - Molecular Mechanisms of Activation and Regulation. Front Immunol. 2015; 6: 262.
73. Bertsias G, Ioannidis JP, Boletis J, Bombardieri S, Cervera R, Dostal C, *et al.* EULAR recommendations for the management of systemic lupus erythematosus. Report of a Task Force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics. Ann Rheum Dis. 2008; 67(2): 195-205.
74. Ruiz-Irastorza G, Ramos-Casals M, Brito-Zeron P, Khamashta MA. Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: a systematic review. Ann Rheum Dis. 2010; 69(1): 20-8.
75. Hogan J, Avasare R, Radhakrishnan J. Is newer safer? Adverse events associated with first-line therapies for ANCA-associated vasculitis and lupus nephritis. Clin J Am Soc Nephrol. 2014; 9(9): 1657-67.
76. Merrell M, Shulman LE. Determination of prognosis in chronic disease, illustrated by systemic lupus erythematosus. J Chronic Dis. 1955; 1(1): 12-32.
77. Pistiner M, Wallace DJ, Nessim S, Metzger AL, Klinenberg JR. Lupus erythematosus in the 1980s: a survey of 570 patients. Semin Arthritis Rheum. 1991; 21(1): 55-64.
78. Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, *et al.* Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. Medicine (Baltimore). 2003; 82(5): 299-308.
79. Doria A, Iaccarino L, Ghirardello A, Zampieri S, Arienti S, Sarzi-Puttini P, *et al.* Long-term prognosis and causes of death in systemic lupus erythematosus. Am J Med. 2006; 119(8): 700-6.
80. Borchers AT, Keen CL, Shoenfeld Y, Gershwin ME. Surviving the butterfly and the wolf: mortality trends in systemic lupus erythematosus. Autoimmun Rev. 2004; 3(6): 423-53.
81. Abu-Shakra M, Urowitz MB, Gladman DD, Gough J. Mortality studies in systemic lupus erythematosus. Results from a single center. II. Predictor variables for mortality. J Rheumatol. 1995; 22(7): 1265-70.

82. Drenkard C, Villa AR, Alarcon-Segovia D, Perez-Vazquez ME. Influence of the antiphospholipid syndrome in the survival of patients with systemic lupus erythematosus. J Rheumatol. 1994; 21(6): 1067-72.
83. Bernatsky S, Ramsey-Goldman R, Clarke A. Exploring the links between systemic lupus erythematosus and cancer. Rheum Dis Clin North Am. 2005; 31(2): 387-402, viii-ix.
84. Kang I, Park S. Infectious complications in SLE after immunosuppressive therapies. Curr Opin Rheumatol. 2003; 15(5): 528-34.
85. Petri M, Perez-Gutthann S, Spence D, Hochberg MC. Risk factors for coronary artery disease in patients with systemic lupus erythematosus. Am J Med. 1992; 93(5): 513-9.
86. Cervera R, Abarca-Costalago M, Abramovicz D, Allegri F, Annunziata P, Aydintug AO, *et al.* Systemic lupus erythematosus in Europe at the change of the millennium: lessons from the "Euro-Lupus Project". Autoimmun Rev. 2006; 5(3): 180-6.
87. Nangaku M, Couser WG. Mechanisms of immune-deposit formation and the mediation of immune renal injury. Clin Exp Nephrol. 2005; 9(3): 183-91.
88. Berden JH, Licht R, van Bruggen MC, Tax WJ. Role of nucleosomes for induction and glomerular binding of autoantibodies in lupus nephritis. Curr Opin Nephrol Hypertens. 1999; 8(3): 299-306.
89. Fries JW, Mendrick DL, Rennke HG. Determinants of immune complex-mediated glomerulonephritis. Kidney Int. 1988; 34(3): 333-45.
90. Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, *et al.* The classification of glomerulonephritis in systemic lupus erythematosus revisited. Kidney Int. 2004; 65(2): 521-30.
91. Cameron JS. Lupus nephritis. J Am Soc Nephrol. 1999; 10(2): 413-24.
92. Smeets B, Kuppe C, Sicking EM, Fuss A, Jirak P, van Kuppevelt TH, *et al.* Parietal epithelial cells participate in the formation of sclerotic lesions in focal segmental glomerulosclerosis. J Am Soc Nephrol. 2011; 22(7): 1262-74.
93. Smeets B, Angelotti ML, Rizzo P, Dijkman H, Lazzeri E, Mooren F, *et al.* Renal progenitor cells contribute to hyperplastic lesions of podocytopathies and crescentic glomerulonephritis. J Am Soc Nephrol. 2009; 20(12): 2593-603.
94. Smeets B, Uhlig S, Fuss A, Mooren F, Wetzels JF, Floege J, *et al.* Tracing the origin of glomerular extracapillary lesions from parietal epithelial cells. J Am Soc Nephrol. 2009; 20(12): 2604-15.
95. Ryu M, Migliorini A, Miosge N, Gross O, Shankland S, Brinkkoetter PT, *et al.* Plasma leakage through glomerular basement membrane ruptures triggers the proliferation of parietal epithelial cells and crescent formation in non-inflammatory glomerular injury. J Pathol. 2012; 228(4): 482-94.
96. McCluskey R. Lupus nephritis. In: Sommers S, Bernstein J, editors. Kidney pathology decennial 1966-1975. New York: Appleton-Century-Crofts; 1975. p. 435-50.
97. Appel GB, Silva FG, Pirani CL, Meltzer JI, Estes D. Renal involvement in systemic lupus erythematosus (SLE). Medicine (Baltimore). 1978; 57: 371-410.
98. Bernatsky S, Boivin JF, Joseph L, Manzi S, Ginzler E, Gladman DD, *et al.* Mortality in systemic lupus erythematosus. Arthritis Rheum. 2006; 54(8): 2550-7.
99. Bertsias GK, Tektonidou M, Amoura Z, Aringer M, Bajema I, Berden JH, *et al.* Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. Ann Rheum Dis. 2012; 71(11): 1771-82.
100. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD, *et al.* American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. Arthritis Care Res. 2012; 64(6): 797-808.
101. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group. KDIGO Clinical Practice Guideline for Glomerulonephritis. Kidney Int Suppl. 2012; 2: 139-274.

102. van Tellingen A, Voskuyl AE, Vervloet MG, Bijl M, de Sevaux RG, Berger SP, *et al.* Dutch guidelines for diagnosis and therapy of proliferative lupus nephritis. Neth J Med. 2012; 70(4): 199-207.
103. Ruiz Irastorza G, Espinosa G, Frutos MA, Jimenez Alonso J, Praga M, Pallares L, *et al.* Diagnosis and treatment of lupus nephritis. Consensus document from the systemic auto-immune disease group (GEAS) of the Spanish Society of Internal Medicine (SEMI) and Spanish Society of Nephrology (S.E.N.). Nefrologia. 2012; 32 Suppl 1: 1-35.
104. Wakasugi D, Gono T, Kawaguchi Y, Hara M, Koseki Y, Katsumata Y, *et al.* Frequency of class III and IV nephritis in systemic lupus erythematosus without clinical renal involvement: an analysis of predictive measures. J Rheumatol. 2012; 39(1): 79-85.
105. Christopher-Stine L, Siedner M, Lin J, Haas M, Parekh H, Petri M, *et al.* Renal biopsy in lupus patients with low levels of proteinuria. J Rheumatol. 2007; 34(2): 332-5.
106. Faurschou M, Starklint H, Halberg P, Jacobsen S. Prognostic factors in lupus nephritis: diagnostic and therapeutic delay increases the risk of terminal renal failure. J Rheumatol. 2006; 33(8): 1563-9.
107. Esdaile JM, Joseph L, MacKenzie T, Kashgarian M, Hayslett JP. The benefit of early treatment with immunosuppressive agents in lupus nephritis. J Rheumatol. 1994; 21(11): 2046-51.
108. Contreras G, Pardo V, Cely C, Borja E, Hurtado A, De La Cuesta C, *et al.* Factors associated with poor outcomes in patients with lupus nephritis. Lupus. 2005; 14(11): 890-5.
109. Wilhelmus S, Bajema IM, Bertsias GK, Boumpas DT, Gordon C, Lightstone L, *et al.* Lupus nephritis management guidelines compared. Nephrol Dial Transplant. 2015.
110. Austin HA, 3rd, Klippel JH, Balow JE, le Riche NG, Steinberg AD, Plotz PH, *et al.* Therapy of lupus nephritis. Controlled trial of prednisone and cytotoxic drugs. N Engl J Med. 1986; 314(10): 614-9.
111. Boumpas DT, Austin HA, Balow JE, Vaughan EM, Yarboro CH, Klippel JH, *et al.* Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. Lancet. 1992; 340(8822): 741-5.
112. Gourley MF, Austin HA, 3rd, Scott D, Yarboro CH, Vaughan EM, Muir J, *et al.* Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis. A randomized, controlled trial. Ann Intern Med. 1996; 125(7): 549-57.
113. Houssiau FA, Vasconcelos C, D'Cruz D, Sebastiani GD, Garrido Ed Ede R, Danieli MG, *et al.* Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. Arthritis Rheum. 2002; 46(8): 2121-31.
114. Appel GB, Contreras G, Dooley MA, Ginzler EM, Isenberg D, Jayne D, *et al.* Mycophenolate Mofetil versus Cyclophosphamide for Induction Treatment of Lupus Nephritis. J Am Soc Nephrol. 2009; 20(5): 1103-12.
115. Ginzler EM, Dooley MA, Aranow C, Kim MY, Buyon J, Merrill JT, *et al.* Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis. N Engl J Med. 2005; 353(21): 2219-28.
116. Henderson L, Masson P, Craig JC, Flanc RS, Roberts MA, Strippoli GF, *et al.* Treatment for lupus nephritis. Cochrane Database Syst Rev. 2012; 12: CD002922.
117. Bruyn GA. Controversies in lupus: nervous system involvement. Ann Rheum Dis. 1995; 54(3): 159-67.
118. Futrell N, Schultz LR, Millikan C. Central nervous system disease in patients with systemic lupus erythematosus. Neurology. 1992; 42(9): 1649-57.
119. Joseph FG, Lammie GA, Scolding NJ. CNS lupus: a study of 41 patients. Neurology. 2007; 69(7): 644-54.
120. Sibley JT, Olszynski WP, Decoteau WE, Sundaram MB. The incidence and prognosis of central nervous system disease in systemic lupus erythematosus. J Rheumatol. 1992; 19(1): 47-52.

121. Wong KL, Woo EK, Yu YL, Wong RW. Neurological manifestations of systemic lupus erythematosus: a prospective study. Q J Med. 1991; 81(294): 857-70.

122. Bertsias GK, Ioannidis JP, Aringer M, Bollen E, Bombardieri S, Bruce IN, *et al.* EULAR recommendations for the management of systemic lupus erythematosus with neuropsychiatric manifestations: report of a task force of the EULAR standing committee for clinical affairs. Ann Rheum Dis. 2010; 69(12): 2074-82.

123. Luyendijk J, Steens SC, Ouwendijk WJ, Steup-Beekman GM, Bollen EL, van der Grond J, *et al.* Neuropsychiatric systemic lupus erythematosus: lessons learned from magnetic resonance imaging. Arthritis Rheum. 2011; 63(3): 722-32.

124. Jennings JE, Sundgren PC, Attwood J, McCune J, Maly P. Value of MRI of the brain in patients with systemic lupus erythematosus and neurologic disturbance. Neuroradiology. 2004; 46(1): 15-21.

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

126. Abbott NJ, Mendonca LL, Dolman DE. The blood-brain barrier in systemic lupus erythematosus. Lupus. 2003; 12(12): 908-15.

127. Johnson RT, Richardson EP. The neurological manifestations of systemic lupus erythematosus. Medicine (Baltimore). 1968; 47(4): 337-69.

128. Ellis SG, Verity MA. Central nervous system involvement in systemic lupus erythematosus: a review of neuropathologic findings in 57 cases, 1955--1977. Semin Arthritis Rheum. 1979; 8(3): 212-21.

129. Sibbitt WL, Jr., Brooks WM, Kornfeld M, Hart BL, Bankhurst AD, Roldan CA. Magnetic resonance imaging and brain histopathology in neuropsychiatric systemic lupus erythematosus. Semin Arthritis Rheum. 2010; 40(1): 32-52.

130. Devinsky O, Petito CK, Alonso DR. Clinical and neuropathological findings in systemic lupus erythematosus: the role of vasculitis, heart emboli, and thrombotic thrombocytopenic purpura. Ann Neurol. 1988; 23(4): 380-4.

131. Hanly JG. Diagnosis and management of neuropsychiatric SLE. Nat Rev Rheumatol. 2014; 10(6): 338-47.

132. McLean BN, Miller D, Thompson EJ. Oligoclonal banding of IgG in CSF, blood-brain barrier function, and MRI findings in patients with sarcoidosis, systemic lupus erythematosus, and Behcet's disease involving the nervous system. J Neurol Neurosurg Psychiatry. 1995; 58(5): 548-54.

133. Fragoso-Loyo H, Atisha-Fregoso Y, Llorente L, Sanchez-Guerrero J. Inflammatory profile in cerebrospinal fluid of patients with headache as a manifestation of neuropsychiatric systemic lupus erythematosus. Rheumatology (Oxford). 2013; 52(12): 2218-22.

134. Lu XY, Zhu CQ, Qian J, Chen XX, Ye S, Gu YY. Intrathecal cytokine and chemokine profiling in neuropsychiatric lupus or lupus complicated with central nervous system infection. Lupus. 2010; 19(6): 689-95.

135. Zandman-Goddard G, Chapman J, Shoenfeld Y. Autoantibodies involved in neuropsychiatric SLE and antiphospholipid syndrome. Semin Arthritis Rheum. 2007; 36(5): 297-315.

136. Ward PA. The harmful role of c5a on innate immunity in sepsis. J Innate Immun. 2010; 2(5): 439-45.

137. Pierangeli SS, Girardi G, Vega-Ostertag M, Liu X, Espinola RG, Salmon J. Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombophilia. Arthritis Rheum. 2005; 52(7): 2120-4.

138. Girardi G, Mackman N. Tissue factor in antiphospholipid antibody-induced pregnancy loss: a pro-inflammatory molecule. Lupus. 2008; 17(10): 931-6.

139. Ten VS, Sosunov SA, Mazer SP, Stark RI, Caspersen C, Sughrue ME, *et al.* C1q-deficiency is neuroprotective against hypoxic-ischemic brain injury in neonatal mice. Stroke. 2005; 36(10): 2244-50.

140. Estes D, Christian CL. The natural history of systemic lupus erythematosus by prospective analysis. Medicine (Baltimore). 1971; 50(2): 85-95.

141. Ginzler EM, Diamond HS, Weiner M, Schlesinger M, Fries JF, Wasner C, *et al.* A multicenter study of outcome in systemic lupus erythematosus. I. Entry variables as predictors of prognosis. Arthritis Rheum. 1982; 25(6): 601-11.

142. Lee P, Urowitz MB, Bookman AA, Koehler BE, Smythe HA, Gordon DA, *et al.* Systemic lupus erythematosus. A review of 110 cases with reference to nephritis, the nervous system, infections, aseptic necrosis and prognosis. Q J Med. 1977; 46(181): 1-32.

143. Feinglass EJ, Arnett FC, Dorsch CA, Zizic TM, Stevens MB. Neuropsychiatric manifestations of systemic lupus erythematosus: diagnosis, clinical spectrum, and relationship to other features of the disease. Medicine (Baltimore). 1976; 55(4): 323-39.

144. Kovacs JA, Urowitz MB, Gladman DD. Dilemmas in neuropsychiatric lupus. Rheum Dis Clin North Am. 1993; 19(4): 795-814.